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Institute of Neuroscience and Medicine
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Kurzzusammenfassung

In der Vergangenheit wurden zahlreiche ^{18}F-markierte aromatische Aminosäuren überwiegend als Radiopharmaka für die Tumordiagnostik mittels Positronen-Emissions-Tomographie entwickelt. Tryptophan erweckte kürzlich große Aufmerksamkeit, da einige Tumorarten im Verdacht stehen, es in erhöhtem Maße aufzunehmen. Diese Aminosäure konnte bisher jedoch nur in unbefriedigender Weise markiert werden. In der Arbeit hier wurde daher eine einfachere nukleophile, dreistufige Radiosynthese zur Darstellung von L-4-[^{18}F]Fluortryptophan entwickelt. Hierzu wurde ein entsprechender carbonyl-aktivierter Markierungsvorläufer mittels Isotopenaustausch radiofluoriert, die aktivierende Carbonyl-gruppe durch reduktive Decarbonylierung entfernt und anschließend durch eine saure Hydrolyse der Schutzgruppen in L-4-[^{18}F]Fluortryptophan überführt.

Zunächst wurde der Einfluss der Positionen des Fluors und der Formylgruppe auf den Isotopenaustausch an diversen Fluor-1*H*-indolcarbaldehyden getestet, welche mit verschiedenen Schutzgruppen am Stickstoff geschützt waren. Weiterhin wurde die Decarbonylierungsreaktion mit $Rh(PPh_3)_3$ an diesen Molekülen untersucht und optimiert. Die besten Ergebnisse zeigte hierbei das 1-Benzyl-4-fluor-1*H*-indol-5-carbaldehyd.

Basierend auf diesen Ergebnissen wurden weiterhin Synthesekonzepte entsprechender Markierungsvorläufer für L-6-[^{18}F]Fluortryptophan und L-4-[^{18}F]Fluortryptophan entwickelt. Damit konnten die Verbindungen Benzyl (2S,5S)-2-tert-butyl-5-[(1-benzyl-4-fluor-5-formyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimidazolidine-1-carboxylat und Benzyl (2S,5S)-2-tert-butyl-5-[(1-Boc-4-fluor-5-formyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimida-zol-idine-1-carboxylat in 11-stufigen linearen Synthesen in Ausbeuten von etwa 8 % erhalten werden. Beide wiesen eine hohe Diastereomerenreinheit von > 99 % auf.

Während die Radiosynthese von L-6-[^{18}F]Fluortryptophan aufgrund der nicht erfolgreichen Hydrolyse der Benzylgruppe scheiterte, konnte L-4-[^{18}F]Fluortryptophan in der dreistufigen Synthese, bestehend aus Isotopenaustausch, reduktiver Decarbonylierung mit $Rh(PPh_3)_3$ und Hydrolyse mit HCl, in einer Enantiomerenreinheit von > 99 % isoliert werden. Nach Optimierung der Radiosynthese gelang die Isolierung des Radiotracers mit einer gesamten radiochemischen Ausbeute von ca. 13 % und einer molaren Aktivität von >70 MBq/mmol bei einer Synthesedauer von etwa 115 min. Somit konnte eine effizientere nukleophile Radiosynthese von L-4-[^{18}F]Fluortryptophan entwickelt werden, welches nun präklinisch evaluiert werden kann.

Abstract

In the past a variety of ^{18}F-labeled aromatic amino acids have been developed, primarily for tumor diagnostics with positron-emission-tomography. Recently tryptophan got high attention, since evidence came up that some tumors exhibit an elevated consumption of it. So far, this amino acid could only be radiofluorinated by unsatisfactory approaches. In the work here, a simpler, 3-step method for a nucleophilic radiosynthesis of L-4-[^{18}F]fluorotryptophan was developed. For this a carbonyl activated precursor was radiofluorinated by isotopic exchange, followed by removal of the activating formyl group by reductive decarbonylation and subsequent hydrolysis of the protecting groups under acidic conditions.

First, the influence of positions of fluorine and of the formyl group on the isotopic exchange was examined in several fluoro-1*H*-indolecarbaldehydes where different protecting groups were attached to the indole nitrogen. Further, a decarbonylation reaction with $Rh(PPh_3)_3$ on those molecules was carried out and optimized. The best results regarding radiochemical yield and chemical stability were obtained with 1-benzyl-4-fluoro-1*H*-indole-5-carbaldehyde.

Based on these results a concept for the synthesis of precursors for L-6-[^{18}F]fluorotryptophan and L-4-[^{18}F]fluorotryptophan was developed. Hereby the compounds benzyl (2S,5S)-2-tert-butyl-5-[(1-benzyl-4-fluoro-5-formyl-1*H*–indol–3-yl)methyl]–3–methyl–4–oxoimidazolidine-1-carboxylate and benzyl (2S,5S)–2–tert–butyl–5-[(1–Boc–4–fluoro–5–formyl-1*H*–indol–3 yl) methyl]-3-methyl-4-oxoimidazolidine-1-carboxylate were prepared following 11-step linear synthetic pathways with overall yields of about 8 % and an enantiomeric purity of > 99 %.

While the radiosynthesis of L-6-[^{18}F]fluorotryptophan was not successful due to the failing hydrolysis of the benzyl group, L-4-[^{18}F]fluorotryptophan was prepared by the three step radiosynthesis, consisting of an isotopic exchange, a reductive decarbonylation with $Rh(PPh_3)_3$ and the hydrolysis of the protecting groups with HCl, yielding an enantiomeric purity of > 99 %. After optimization of this procedure L-4-[^{18}F]fluorotryptophan was isolated in a radiochemical yield of ca. 13 % and a molar activity of > 70 MBq/mmol within about 115 min. Hence, a new and more efficient nucleophilic radiosynthesis of L-4-[^{18}F]fluoro-tryptophan was developed which is now available for preclinical evaluation.

Contents

1. Introduction

In recent years, the field of radiopharmacy and radiochemistry gained an increased impact. This was possible through the findings of Röntgen and Becquerel who discovered radioactivity at the end of the 19th century by the observation of x-rays and α-radiation, respectively.

First medicinal trials with radiation were made with skin disorders, tuberculosis or epilepsy.[1] A few years after the discovery of radiation it was already used for the treatment of tumors with promising results. Irradiation itself was usually carried out by implantation of radiumbromide capsules since this made the removal after treatment easy.[2]

In 1898, the harmful properties of radiation towards microorganisms were found after the successful treatment of Morbus Lupus by irradiation. Between 1918 and 1930 radioactivity attained an enormous popularity. At this time it was believed that radiation has rejuvenating and curing effects. Therefore, a variety of radium containing products such as water or chocolate were commercially available to everyone.[2]

The first application of radioactivity as a tracer began with de Hevesy and Paneth who used radioactive nuclides for isotopic exchange reactions, diffusion studies and also first in life sciences for the determination of the uptake of lead in different types of plants.[3]

They are usually called the inventors of the “tracer-concept”. This concept is based on small amounts of a radiolabeled substance which is then used as a “mass substitute”. By application into an organism, the pathway and distribution of this substance can be measured due to the low threshold of radioactivity. The very low amounts of tracer, that are used for this purpose do normally not have any impact on physiology and can be neglected in most cases. De Hevesy was awarded with the Nobel prize in chemistry in 1933 for the application of this principle *in vivo*.

The requirement for a widespread application of this principle was the development of artificial radioactivity. This was done in 1929 by Lawrence who built the first cyclotron at the University of California, Berkeley. The development of this new technique was delayed through the Second World War, especially in Germany.

However, the evolution of radiopharmacy as an independent research field began after the world war. The first radionuclides for medicinal applications were developed in 1945 at Oak Ridge (Tennessee) and the first radiopharmaceutical products such as $Na^{131}I$ were available in

1955. In the middle of the 70s the *Section on Nuclear Pharmacy* was founded by the *American Pharmaceutical Association*.[2] Since then, uncountable developments have been achieved in this field and their number is still growing. Nowadays, radiotracers are mainly used for diagnosis and therapy in nuclear medicine, but also for a broad variety of research purposes.

1.1 Principle of positron emission tomography (PET)

Positron Emission Tomography (PET) is an analytical imaging technology in nuclear medicine using labeled compounds as molecular probes to measure biochemical processes *in vivo*.[4] It is mainly applied for diagnosis in nuclear medicine but also for research purposes in the fields of oncology[5],[6],[7],[8], neurology[9],[10],[11], cardiology[12],[13],[14] and psychiatry[15]. Other imaging techniques such as magnetic resonance imaging (MRI), x-rays or ultrasound, that are commonly used for anatomical or structural imaging, give limited or no information on biochemical and molecular processes. Therefore, these imaging techniques are limited to the *in vivo* measurement of diseases associated with structural changes whereas PET and SPECT (single photon emission tomography), a technique related to PET but relying on the detection of a single photon, are great options for the *in vivo* measurement of malfunctions related to metabolic events.

PET is based on the simultaneous detection of two 511 keV photons resulting from the vice versa annihilation of a positron and an electron. Positron emitters are unstable isotopes bearing an excess of protons in their nucleus. The nucleus of a positron emitter stabilizes by emission of a positron (β^+) and a neutrino (Scheme 1) which is formally the conversion of a proton into a neutron. The emitted positron thermalizes through collisions with other atoms and molecules in its surrounding.

$$ {}^{A}_{Z}X_N \rightarrow {}^{A}_{Z-1}Y_N + {}^{0}_{1}\beta^+ + \nu_e $$

Scheme 1 Decay of a radionuclide by positron emission.

After thermalization a *para*-positronium, a hydrogen like particle consisting of an electron and a positron is formed in its singulet state (S = 0). The positron and the electron are anti-particles that eventually annihilate and emit two 511 keV γ-rays (annihilation radiation) in an

angel of almost 180°. Compared to the mainly occurring *para*-positronium the *ortho*-positronium with parallel spins (S = 1) is relatively rare.[16] In contrast to the β^+-particles the emitted γ-radiation is body penetrating and can be detected by special szintillation crystals outside the body. Common materials for those detectors are high density crystals with a good ability to absorb the emitted γ-radiation such as bismut-germanate (BGO)[17] and Ce^{3+} doped lutetium-yttrium-oxide-ortho-oxosilicate (LSO)[18]. If two γ-photons with an energy of 511 keV are detected in two detectors with an 180° angle within a certain time window of generally less than ten nano seconds, the signal is registered as valid (see Figure 1). The position of the annihilation can then be assigned along the connecting line in between the two events. This is a major advantage of PET over SPECT because the noise signal of other occurring events is diminished. The high spatial resolution of PET results from the fact that it is only dependent on the distance travelled by the positron and defined by the kinetic energy of this particle, which usually reaches from hundreds of keV up to a few MeV and results in a travelling distance of up to a few millimeters in human tissue.[19]

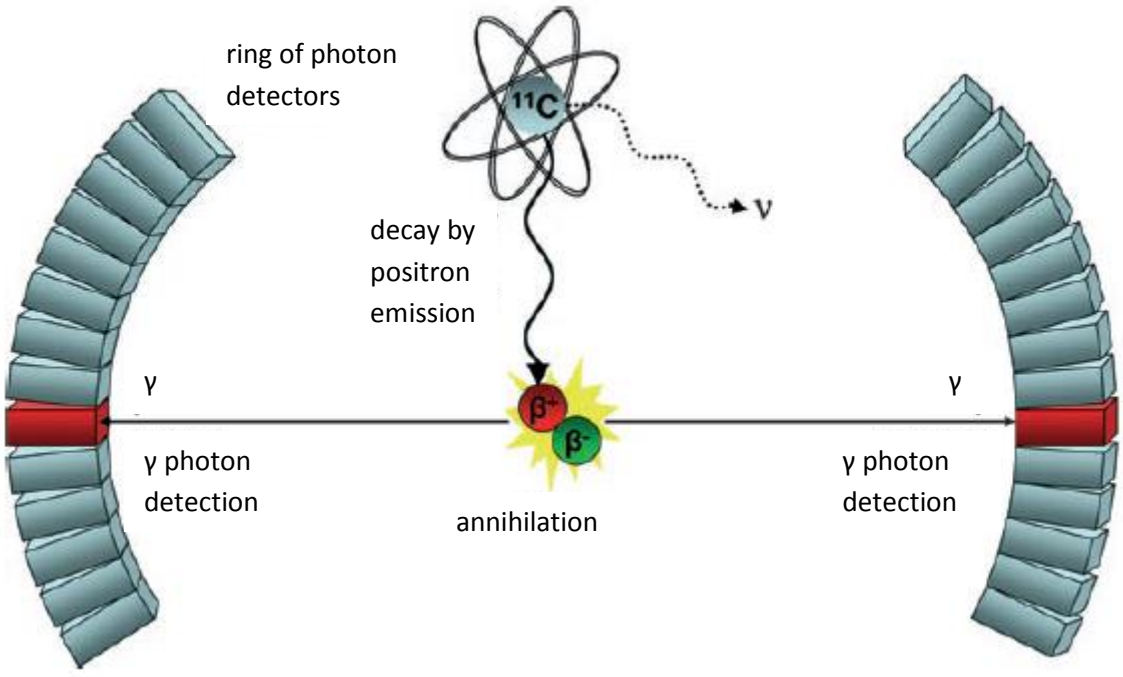


Figure 1 Schematic representation of the principle of PET showing positron decay and γ-annihilation which produces two γ-photons of 511 keV.[20]

In order to obtain reconstructed images that are linearly correlated to the measured activity several corrections need to be done to avoid uncertainties caused by scattering, attenuation

and the dead time of the detectors. For this purpose usually a transmission measurement is done before the radiotracer is injected. This is generally accomplished by use of the positron emitter ^{68}Ga. Thereby one of the annihilation photons is used for the transmission information and the other one is needed to define the line of response. The application of this procedure allows the quantification of the radioactivity measured in the tissue which is the greatest advantage of PET.[21] In order to gain quantitative medical relevant information from the quantitative measurement of the distribution of the radioactivity in the body, the radioactivity is measured, which represents the distribution of the radiotracer *in vivo*. These data are then combined with a suitable bio-mathematical compartment model that describes the pharmacokinetic behavior of the radiopharmaceutical in the human body and thereby delivers quantitative information about the process under study.[22] In a typical PET study, a positron labeled probe is injected, and PET scans provide measures of the tissue concentration of the probe and its metabolites over time. These data are combined with a measurement of the plasma probe concentration over the course of time and, thus, representing its delivery to tissue. The obtained data are subsequently processed with the help of a suitable compartmental model and differential equations, which describe the transport and reaction processes the probe undergoes. Thus, an image of the physiological process of interest can be created. With the help of this image further analysis and rating of this process become possible.[4] Since PET probes are usually administered in the concentration ranges of pico- to femtomoles it is unlikely they have a pharmacodynamic influence on the biological process they are involved in.

Some of the commonly used short lived radioisotopes for PET are identical with those found in biologically active substances. Hence it is possible to label these molecules without changing their original composition or structure. The widely used so called “organic” PET isotopes including their half-life, production route and decay product are listed in Table 1.[20] The isotopes shown in Table 1 all have relatively short half-lives which leads to one of the main challenges for radiochemists because the labeled probe needs to be synthesized, purified, analyzed, and formulated within a time scale of roughly two or three half-lives.[19] Therefore, the development of rapid synthetic strategies for the production of these radio-labeled products is of high interest.[20]

Nowadays PET measurements are usually combined with anatomical measurements by a CT (computed tomography) or MRI. Hereby both techniques are normally combined and integrated into one device. This allows matching of the functional data obtained by PET with the anatomical data recorded by CT or MRI and thereby supports diagnosis.

Table 1 Commonly used short-lived radionuclides in PET with their half-lifes, nuclear reactions, target and decay products.[12]

Radionuclide	$T_{1/2}$ [min]	Nuclear reaction	Target material	Product	Decay product
^{11}C	20.4	$^{14}N(p,\alpha)^{11}C$	N_2 (+O_2) N_2 (+H_2)	[^{11}C]CO_2 [^{11}C]CH_4	^{11}B
^{13}N	9.97	$^{16}O(p,\alpha)^{13}N$	H_2O H_2O + EtOH	[^{13}N]NOx [^{13}N]NH_3	^{13}C
^{15}O	2.04	$^{14}N(d,n)^{15}O$	N_2 + O_2	[^{15}O]O_2	^{15}N
^{18}F	110	$^{20}Ne(d,\alpha)^{18}F$ $^{18}O(p,n)^{18}F$	Ne (+F_2) H_2[^{18}O]O	[^{18}F]F_2 [^{18}F]F^-	^{18}O

1.2 Labeling of organic molecules with fluorine-18

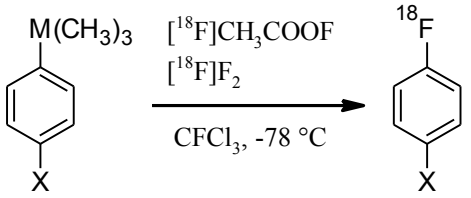
Fluorine-18 is the most widely used radionuclide for routine diagnosis in PET. It was first produced in 1936[23] and found its way into medical application during a few years. The intensive use of fluorine-18 can be dedicated to its almost perfect nuclear properties. It also can be produced in high yields even on low energy cyclotrons which guarantees its availability quite easily. The relatively long half-life of 109.7 min allows shipping of the desired products within a few hours to medical institutes that do not have a cyclotron on site. The low maximum kinetic energy of the positron emitted during the nuclear decay of 635 keV (mean energy of 250 keV) results in a maximum range in water of 2.4 mm (mean range of 0.7 mm).[24] Thus, the low β^+-energies enable high resolution PET images and relatively low radiation doses to the patients.

Most of the established labeling methods with fluorine-18 follow general fluorination concepts applied in organic chemistry like electrophilic or nucleophilic substitution. But the short half-life and the sub-nanomolar amount of fluorine-18 used under no-carrier-added (n.c.a.) conditions cause difficulties in some reactions like the Balz-Schiemann reaction. Therefore, radiofluorination reactions do often require special methods and synthesis-techniques that make use of non-standard leaving groups and reaction set-ups.

1.2.1 Electrophilic fluorination

For electrophilic ^{18}F-fluorination, [^{18}F]F_2 is essential which is nowadays generally produced by the ^{18}O(p,n)^{18}F nuclear reaction on a ^{18}O-gas target. After irradiation a small amount of carrier F_2 is added to the target to remove the produced radioactivity.[25] The necessary addition of carrier and the fact that every molecule of [^{18}F]F_2 carries only one radioactive atom result in a maximum radiochemical yield of 50 % and a low specific activity. Hence radiotracers produced by electrophilic radiofluorination are limited to applications where a high specific activity is not required e.g. ^{18}F-labeled amino acids or [^{18}F]fluorosugars.

Another major drawback of the electrophilic ^{18}F-fluorination is the high reactivity of [^{18}F]F_2 which results in a low selectivity, a broad variety of undesired side products and a need of extensive purification. In order to increase the regioselectivity in arenes, demetallation procedures were used (Scheme 2) with [^{18}F]F_2 and [^{18}F]CH_3COOF as fluorination agents.[26],[27]

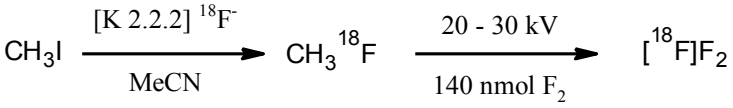


Scheme 2 Regioselective ^{18}F-labeling via electrophilic demetallation reactions (M = Sn, Ge, Si; X = OCH_3, CH_3, H, F, CF_3, NO_2).[19]

Experiments performed on a series of *para*-substituted trimethylaryltin, -germanium, -and silicon compounds gave the corresponding [^{18}F]fluoroarenes in high regioselectivity showing a decrease in the radiochemical yield in the order Sn > Ge > Si including rings containing electron withdrawing groups. Only marginal differences could be observed between [^{18}F]F_2 and the somewhat less reactive CH_3COO[^{18}F]F.[24]

Attempts were also made in order to increase the specific activity of [^{18}F]F_2 and to reduce the amount of carrier needed. Besides electrochemical attempts for this reason[28], methyl[^{18}F]fluoride was prepared by no-carrier-added (n.c.a.) nucleophilic substitution of CH_3I with n.c.a. [^{18}F]fluoride followed by an electric gaseous discharge (Scheme 3). This method allows the production of [^{18}F]F_2 with specific activities of up to 30 GBq/µmol, but has

a major drawback on the radiochemical yield. Further, the specific activity is still not high enough for, e.g. labeling of receptor-ligands. [29]



Scheme 3 Elemental [^{18}F]fluorine generated by electric discharge of CH_3[^{18}F]F.[29]

1.2.2 Nucleophilic fluorination

The most important method for labeling of organic molecules with fluorine-18 is the nucleophilic substitution with n.c.a. [^{18}F]fluoride easily available with both, high activity and high specific activity via the ^{18}O(p,n)^{18}F nuclear reaction on H_2[^{18}O]O enriched targets. The [^{18}F]fluoride is received from the target in aqueous solution and, due to high hydration, is poorly reactive. Further treatment of the [^{18}F]fluoride containing solution is necessary prior to any use in nucleophilic radiosyntheses. Although amply described[30],[31] it should be briefly summarized here: The first step herein is usually the separation of [^{18}F]fluoride from the expensive ^{18}O-enriched water. This generally takes place by adsorption of [^{18}F]fluoride on an ion exchange resin, enabling the recovery of the ^{18}O-enriched water, followed by the elution of the [^{18}F]fluoride with a small amount of an aqueous solution of a weak base.[32],[33] The remaining water is removed by repeated azeotropic distillation with MeCN.[31]

Alternatively, [^{18}F]fluoride can be separated by use of an electrochemical cell. This method does not require thermal drying. After adsorption the electrode is rinsed with a dry organic solvent to remove the remaining water. Afterwards the [^{18}F]fluoride is released into an aprotic polar solvent of choice by inversion of the voltage in the cell and eluted.[34]

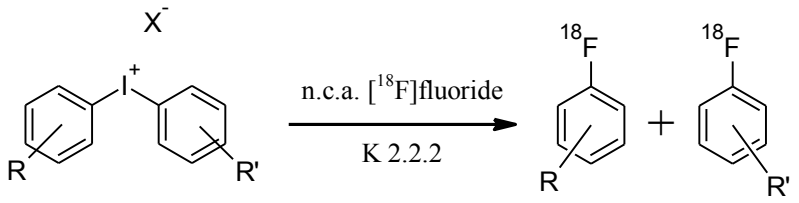
Because of the strong hydration of fluoride in water, labeling has to take place under aprotic conditions. Therefore, routine labeling via nucleophlic substitution is usually performed in dipolar aprotic solvents such as DMSO (dimethylsulfoxide), DMF (*N,N*-dimethylformamide) or MeCN (acetonitrile) using alkali salts with soft cations resulting in weak and easy to separate ion pairs and “naked” fluoride ions of high nucleophilicity. Additional anion activation can be achieved by the use of phase transfer catalysts (PTC) like tetraalkylammonium carbonates[32],[35] or aminopolyether like the mainly used Kryptofix®2.2.2 in combination with alkali carbonates or oxalates.[36],[37],[38] This is the

generally favored system in most n.c.a. radiofluorination reactions. Also wall loss of the [^{18}F]fluoride plays a minor role if the solubility product of the cryptate complex is not exceeded.[39] However, there are a few cases where tetrabutylammonium carbonate shows advantages over the Kryptofix® system.[30],[33]

Since direct aliphatic ^{18}F-fluorinations are generally performed in dipolar aprotic solvents, they proceed according to an S_N2-mechanism. Good leaving groups such as mesylates, triflates or other sulfonic esters are required for high radiochemical yields. The yields are also increasing from tertiary to primary carbon position.

For aromatic nucleophilic radiofluorination (S_NAr) the aromatic ring needs to be activated by an electron withdrawing group like an aldehyde, ketone or nitrile in *ortho* or *para* position to the leaving group. Commonly used leaving groups for this type of reaction are -F, -NO_2 or -$N(CH_3)_3^+$. In general aromatic nucleophilic substitution reactions require higher temperatures than aliphatic ones. Hence solvents with higher boiling points such as DMF or DMSO are preferred here. The application of those labeling methods has been reviewed in detail by Ermert and Coenen.[40]

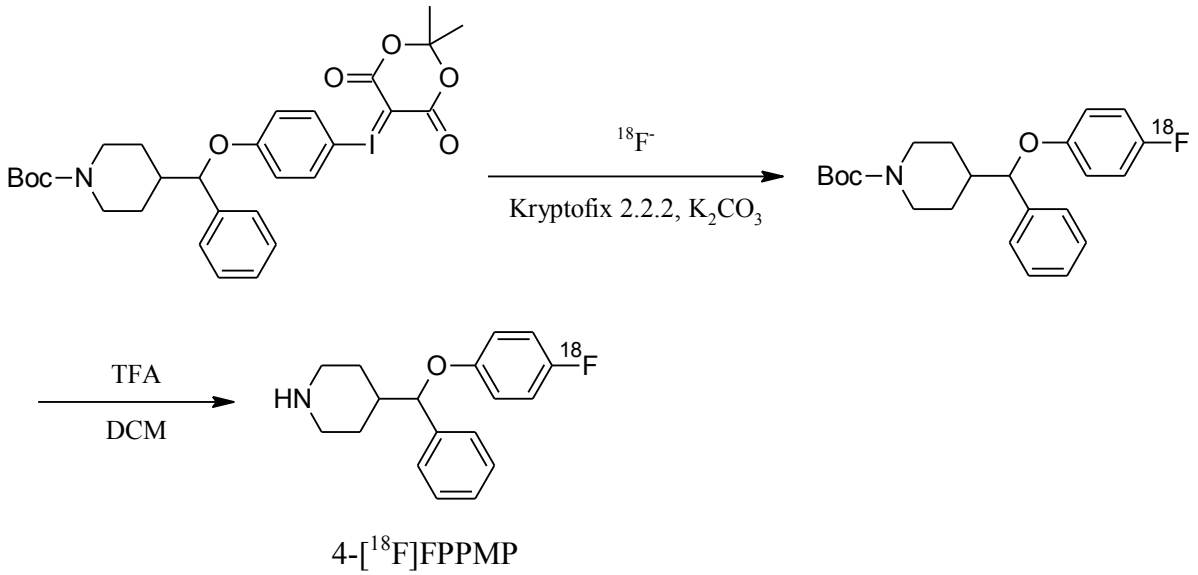
Further, the requirement of electron withdrawing groups limits the scope of direct aromatic fluorination. Detailed information about this can be found in a review by Coenen and Ermert.[41] For the fluorination of an electron rich arene it is generally essential to generate an electrophilic center. Diaryliodonium salts offer a promising possibility for this type of reaction. It is well known that they react with a wide range of nucleophiles giving the corresponding arenes and iodoarenes.[42] The distribution of the resulting products after the nucleophilic attack is strongly dependent on the electronic and steric character of each arene ring (Scheme 4). The reaction itself proceeds also via a S_NAr-mechanism and in asymmetric diaryliodonium salts the more electron deficient ring is of course preferred for the nucleophilic attack of the [^{18}F]fluoride.



Scheme 4 General principle of ^{18}F-labeling via diaryliodonium salts (X = Cl, Br, TsO, ClO_4, CF_3SO_3; R, or R' = H, Cl, Br, I, NO_2, CH_3, OCH_3.

Also symmetrical iodonium salts have been prepared in order to avoid ^{18}F-labeled side products originating from ^{18}F-fluorination of the undesired arene ring.[43],[44],[45]

Due to their chemical properties the use of iodonium salts was limited mainly to small molecules or synthons in the past. Recently, aryliodonium ylides were found to be a promising alternative for direct n.c.a. radiofluorination of complex, even electron rich [^{18}F]fluoroarenes. An example for this is the radiofluorination of the NET (norepinephrine transporter) and SERT (serotonin transporter) ligands 3- and 4-fluorophenoxy)phenyl-methyl)piperidine (3- and 4- FPPMP) via the corresponding iodonium ylides giving the transporter ligands in a RCY of up to 45 % (Scheme 5).

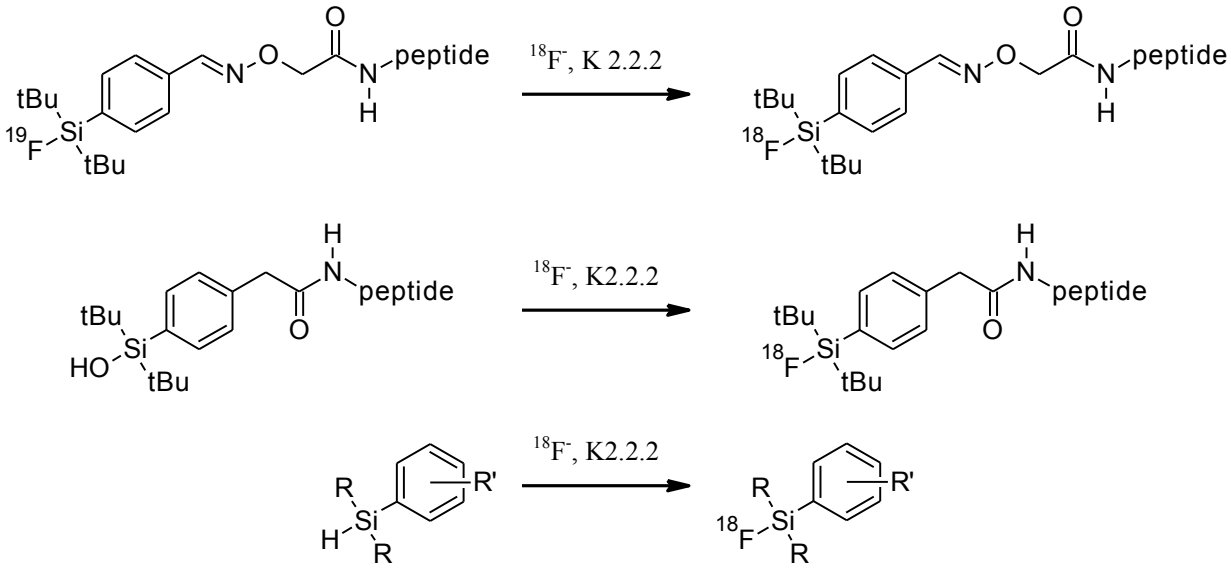


Scheme 5 Radiosynthesis of 4[^{18}F]FPPMP via the corresponding iodonium ylide.[46]

However, when the radiosynthesis of 4-[^{18}F]FPPMP was carried out, also the positional isomer 3-[^{18}F]FPPMP was always detected with about 10 % RCY. This behavior was also observed with the small molecules 4-methoxyphenyl-(5-[2,2-dimethyl-1,3-dioxane-4,6-dione])ylide and 4-benzyloxyphenyliodonium-(5-[2,2-dimethyl-1,3-dioxane-4,6-dione])ylide indicating rearrangement reactions during substitution.[46]

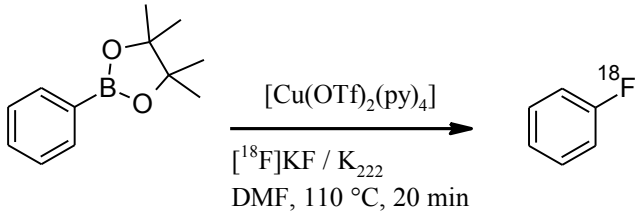
As the incorporation of fluorine-18 by nucleophilic substitution into an organic compound at a carbon atom is usually carried out under rather harsh conditions like high temperature and basic conditions, the labelling of complex biomolecules (e.g. peptides or proteins) is often not possible due to their limited stability in this process. Recently, the high affinity of fluorine to silicone was used in order to accomplish ^{18}F-fluorination under very mild conditions. With this principle basically two approaches were made for the introduction of fluorine-18. The

preparation of a synthon by an isotopic ^{18}F-for-^{19}F exchange[47] that is only applicable when high specific activities are not required, and a method where the silicone is directly attached to the desired molecule. In the second case the leaving group on the silicone is usually an alkoxy, hydroxy or hydride group.[48][49] Examples for the application of these methods are shown in Scheme 6.



Scheme 6 Examples for the radiofluorination of silicone precursors with fluorine-18 by isotopic ^{18}F-for-^{19}F exchange, ^{18}F-for-OH substitution and ^{18}F-for-H-substitution.

Another ^{18}F-fluorination method for the radiolabeling of arenes that was recently introduced is based on boronic esters. One of the major advantages of this procedure is that the arenes do not require previous activation by an electron withdrawing group which is essential when radio-fluorination is carried out under standard S_NAr conditions. Herein, a direct copper(II)-mediated fluorination of aromatic boronic esters with n.c.a. [^{18}F]fluoride has been accomplished using a commercially available copper(II)-catalyst (Scheme 7). The boronic esters herein are usually prepared by a transition metal catalyzed boron for halogen exchange. Using this procedure a broad variety of small arenes has been labeled in moderate to good RCY.[50]



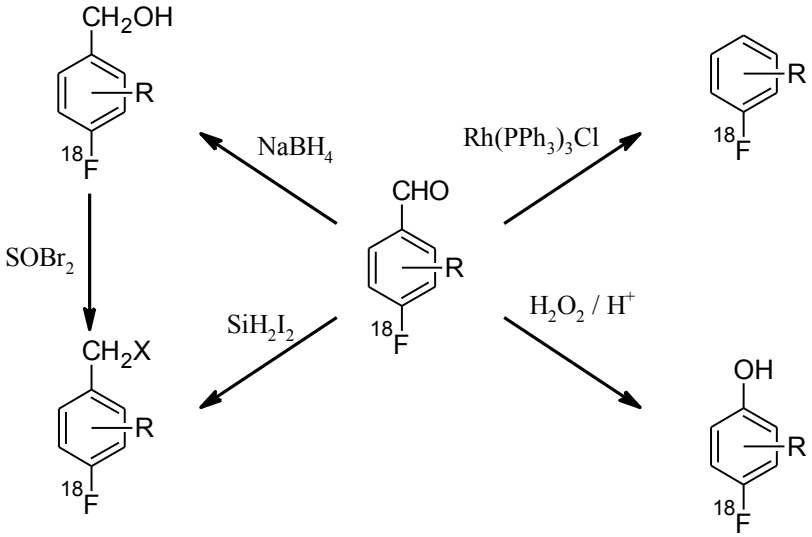
Scheme 7 Aromatic Cu(II)-mediated ^{18}F-fluorination of pinacol-derived boronic esters with n.c.a. [^{18}F]fluoride.[50]

An interesting method that has emerged in recent years is the radiofluorination of peptides by the chelation of $Al^{18}F$. The best RCY and *in vivo* stability were obtained when pentadentate ligands were applied leaving one binding site of aluminum free for the coordination with [^{18}F]fluoride. For this reason, a good ligand is 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), where one of the carboxylic acids is already linked to the peptide of interest. By the application of this labeling technique a RCY of up to 97 % and specific activities of up to 115 GBq/µmol were obtained within a very short reaction time of about 115 min. Since then this method has been used for the radiofluorination of several proteins and peptides, especially RGD peptides.[51]

1.2.3 Fluorination through secondary groups

Radiofluorination is not always possible by a direct one-step labelling procedure with [^{18}F]fluoride. This is especially true when electron rich arenes and macromolecules such as peptides or proteins are subject to labeling. Therefore, alternative procedures are required that allow nucleophilic radiofluorination of those molecules.

This goal can be achieved using a secondary group that can be directly labeled through nucleophilic substitution with n.c.a. [^{18}F]fluoride followed by coupling to the target molecule.

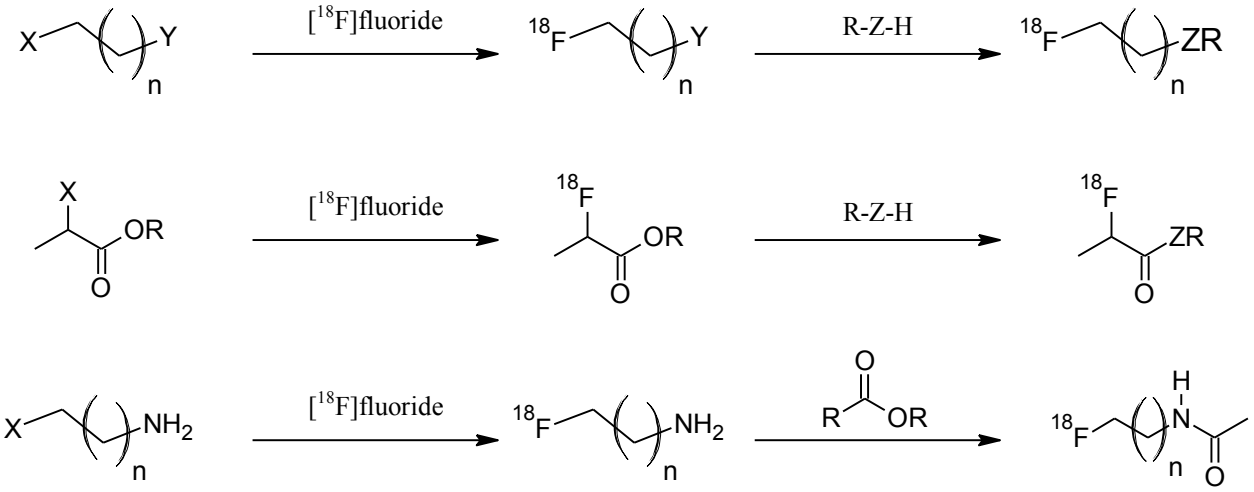


Scheme 8 Possibilities of conversion of [^{18}F]fluorobenzaldehydes as examples of versatile synthons.[52]

Aromatic, so called radiofluorinated synthons are often derived from 2- or 4-[^{18}F]fluorobenz-aldehydes that are easily accessible by direct labeling with n.c.a. [^{18}F]fluoride. The activating

aldehyde in those molecules can be converted to versatile functional groups, e.g. benzylhydroxides or –halides by reductive reactions. It is also possible to remove the aldehyde function completely by reductive decarbonylation. Conversion to phenols can be accomplished by the Baeyer-Villiger Oxidation. Some important aromatic synthons derived from [^{18}F]fluorobenzaldehydes are shown in Scheme 8. More detailed information on this can be found in the review by Ermert and Coenen.[52],[30][30][30][30]

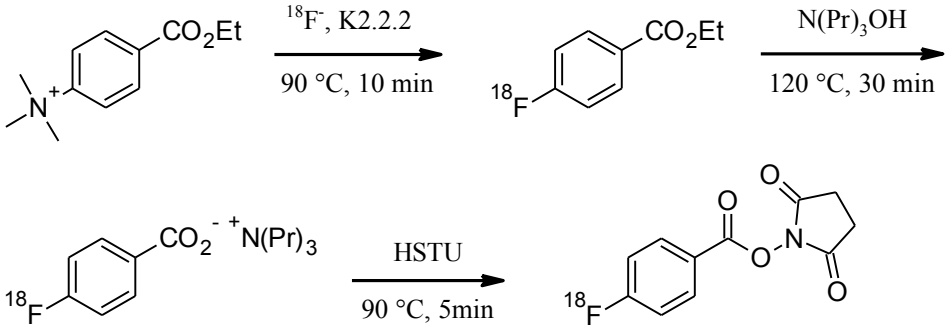
Other important procedures for the introduction of [^{18}F]fluoride are the ^{18}F-fluoroalkylation, the ^{18}F-fluoroacylation and the ^{18}F-fluoroamidation (Scheme 9).[53],[54],[55] Applications for the prosthetic groups shown in Scheme 9 are widespread and reach from radiolabeling of macromolecules and peptides to ^{18}F-fluorination of small molecules such as neurotransmitters or hormones. By far the most important of these procedures is the fluoroalkylation which was used extensively in the recent years to radiofluorinate molecules like tyrosine, L-DOPA or L-5-hydroxytryptophan. The ^{18}F-fluoroalkylation synthons are usually prepared by ^{18}F-fluorination of symmetrically 1,n-substituted precursors. The most commonly used of those ^{18}F-fluoroalkylation agents is probably [^{18}F]fluoroethyltosylate which has been applied to a series of radiolabeled compounds.[56],[57],[58]



Scheme 9 Prosthetic groups for ^{18}F-fluoroalkylation, ^{18}F-fluoroacylation and ^{18}F-fluoro-amidation (X, Y = Br, I, OTs, OTf; Z = N, O, S; R = alkyl, aryl).[53][54][55]

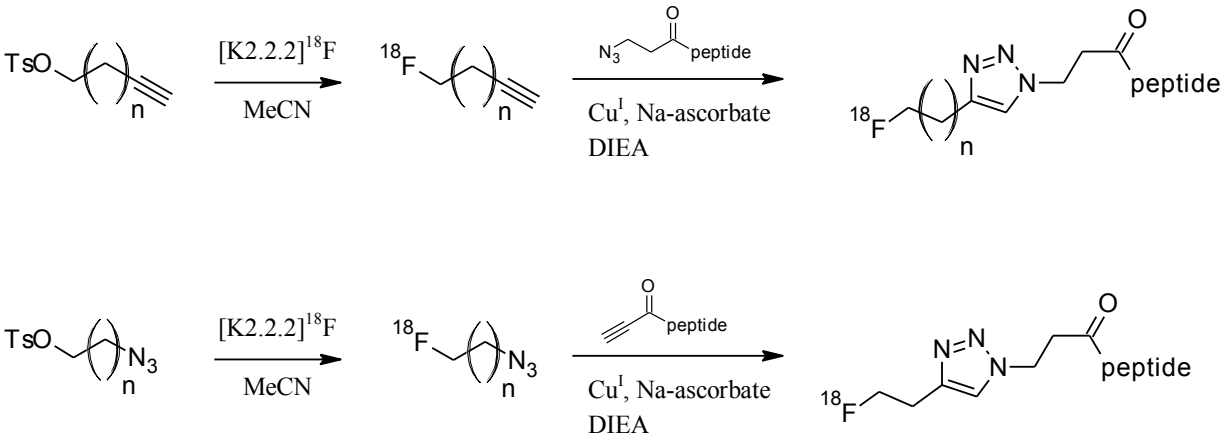
A further important secondary group that is mainly used for protein or peptide labeling is *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB). This versatile compound can be prepared in a three step linear radiosynthesis in less than 60 min. The starting material is ethyl 4-(trimethylammonium triflate)benzoate that is radiofluorinated in the first step followed by

hydrolysis of the ester and subsequent coupling with *N,N,N',N'*-tetramethyl-*O*-(*N*-succinimidyl)uronium hexafluorophosphate (HSTU) giving a RCY of about 44 %.[59] The synthesis is shown in Scheme 10.



Scheme 10 Synthetic route for the preparation of [^{18}F]SFB in a three step linear pathway in less than 60 min.[59]

The radiosynthesis of [^{18}F]SFB was later optimized using a microwave assisted setup. Thereby the synthesis time could be reduced to 20 min while increasing the RCY to about 61 %.[60] Labeling with [^{18}F]SFB is usually done at amine functions of amino acids and proteins.



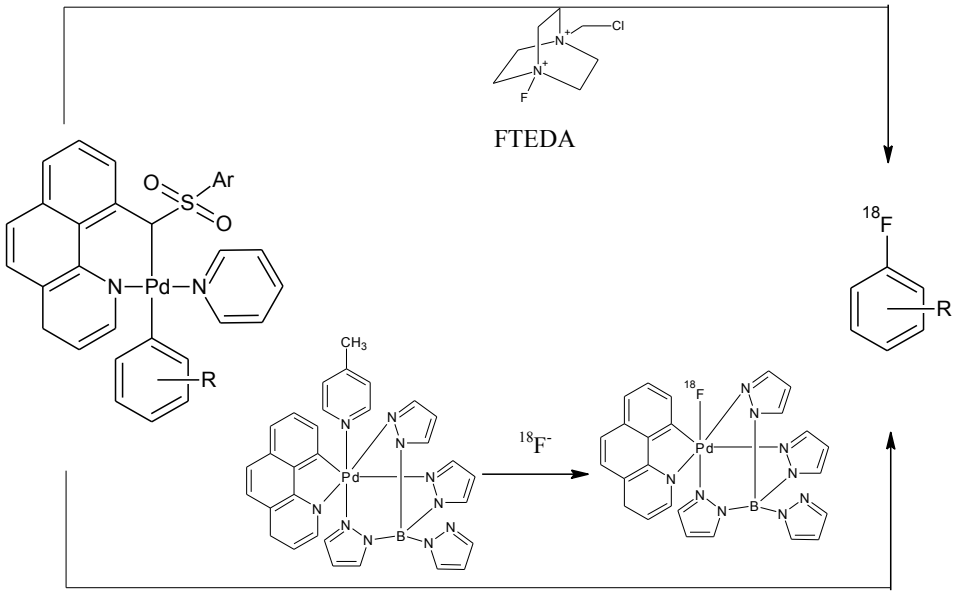
Scheme 11 The two alternative onsets for Huisgen´s 1,3-dipolar cycloaddition for preparation of [^{18}F]fluoropeptides.[61],[62]

An additional concept that has become popular in the past few years is the ^{18}F-fluorination via "click chemistry". Most often, a Huisgen 1,3-bipolar cycloaddition of an alkyne and an azide is used for this purpose (Scheme 11). The reaction originally required the addition of Cu^{I} and proceeds under mild conditions in aqueous solution. In the past the addition of Cu^{I} was a problem when the radiotracer should be used for *in vivo* applications, but in recent years several optimizations for this type of reaction were made, that allow radiofluorination by this

method without the addition of Cu^{I}. Some examples for this can be found in the literature.[63][64] Further advantages are that generally there is no need for protection of functional groups and that the reaction is usually done within 30 min giving high RCY.[62][61]

1.2.4 Transition metal catalyzed fluorination

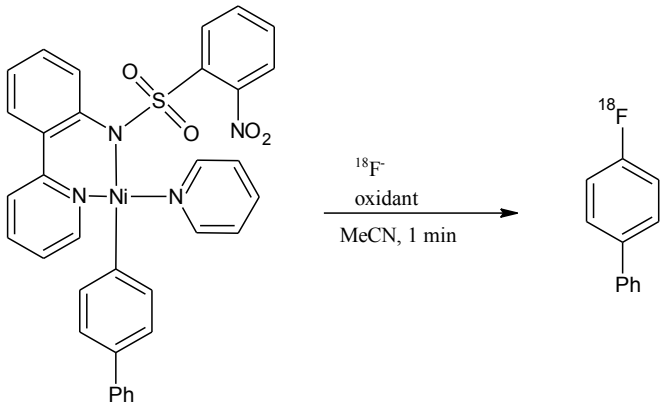
In recent years methods relying on transition metal catalyzed mechanisms came into focus of radiochemists. Since then a variety of approaches has been made in this direction. One of the first approaches in this direction was made by Lee et al.[65] who developed a Pd-catalyzed electrophilic late stage ^{18}F-fluorination method starting from n.c.a. [^{18}F]fluoride (see Scheme 12). The major drawback of this method is the preparation of the palladium complex which is hard to facilitate and requires extremely anhydrous conditions.



Scheme 12 Electrophilic radiofluorination of palladium aryl complexes to afford aryl fluorides. Top: With the electrophilic fluorination reagent F-TEDA. Bottom: With the Pd(IV) fluoride complex, made from [^{18}F]fluoride.[65]

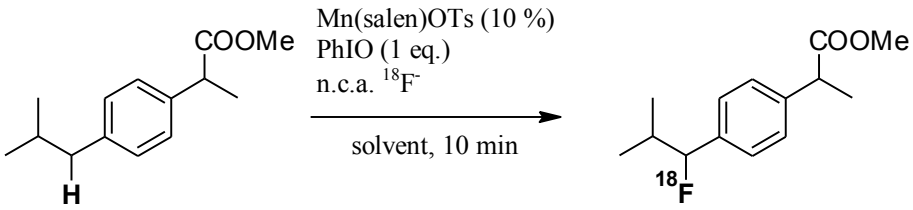
An improved modification of this method was developed relying on a Ni-mediated ^{18}F-fluorination with less sensitive complexes and a higher RCY (see Scheme 13). The fluorination is accomplished by combination of a corresponding Ni-complex with an oxidant and aqueous [^{18}F]fluoride. This method avoids some of the limitations inherent to the

palladium chemistry shown in Scheme 12, especially the possibility of a one-step radiosynthesis. Furthermore, the Ni-complex is much more stable. The drawback of this procedure is the requirement of a special oxidant that is very sensitive to humidity and needs to be prepared and handled under extremely anhydrous conditions in glove-boxes.[66]



Scheme 13 Ni-mediated nucleophilic n.c.a. ^{18}F-fluorination of simple aromatic compounds.[66]

Recently, a method was developed which describes a late stage benzylic ^{18}F-for-H fluorination with n.c.a. [^{18}F]fluoride based on Mn(salen)-complexes (see Scheme 14). It proved suitable for a large series of small arenes such as the pharmaceutical Ibuprofen.[67]



Scheme 14 Benzylic ^{18}F-for-H fluorination with n.c.a.[^{18}F]fluoride. [67]

1.3 Indoles

The indole motive is one of the most frequently occurring heterocyclic moieties in natural products.[68] It is, for example, part of the essential amino acid L-tryptophan, its metabolite skatol, the plant growth factor 3-indolylacetic acid (heteroauxin) and the neurotransmitter serotonin.[69] There is also a broad variety of plant alkaloids that contain indole, e.g. strychnine and ellipticin. Many of those plant alkaloids are highly toxic but may have properties that are highly valuable for special medicinal applications.[70] Indole was first found

by Baeyer and Knoop while studying the structure and properties of indigo.[71] In 1869 Baeyer and Emmerling proposed the structure that is still generally accepted (Figure 2).[72]

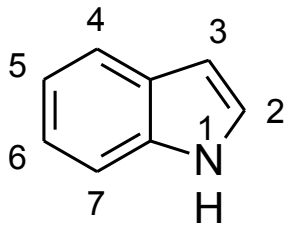
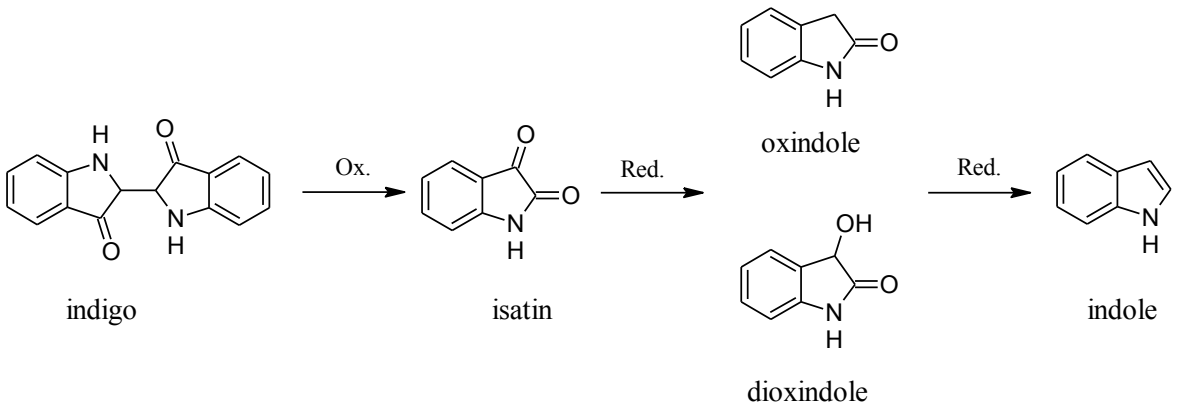


Figure 2 Chemical structure of indole with IUPAC numbering.

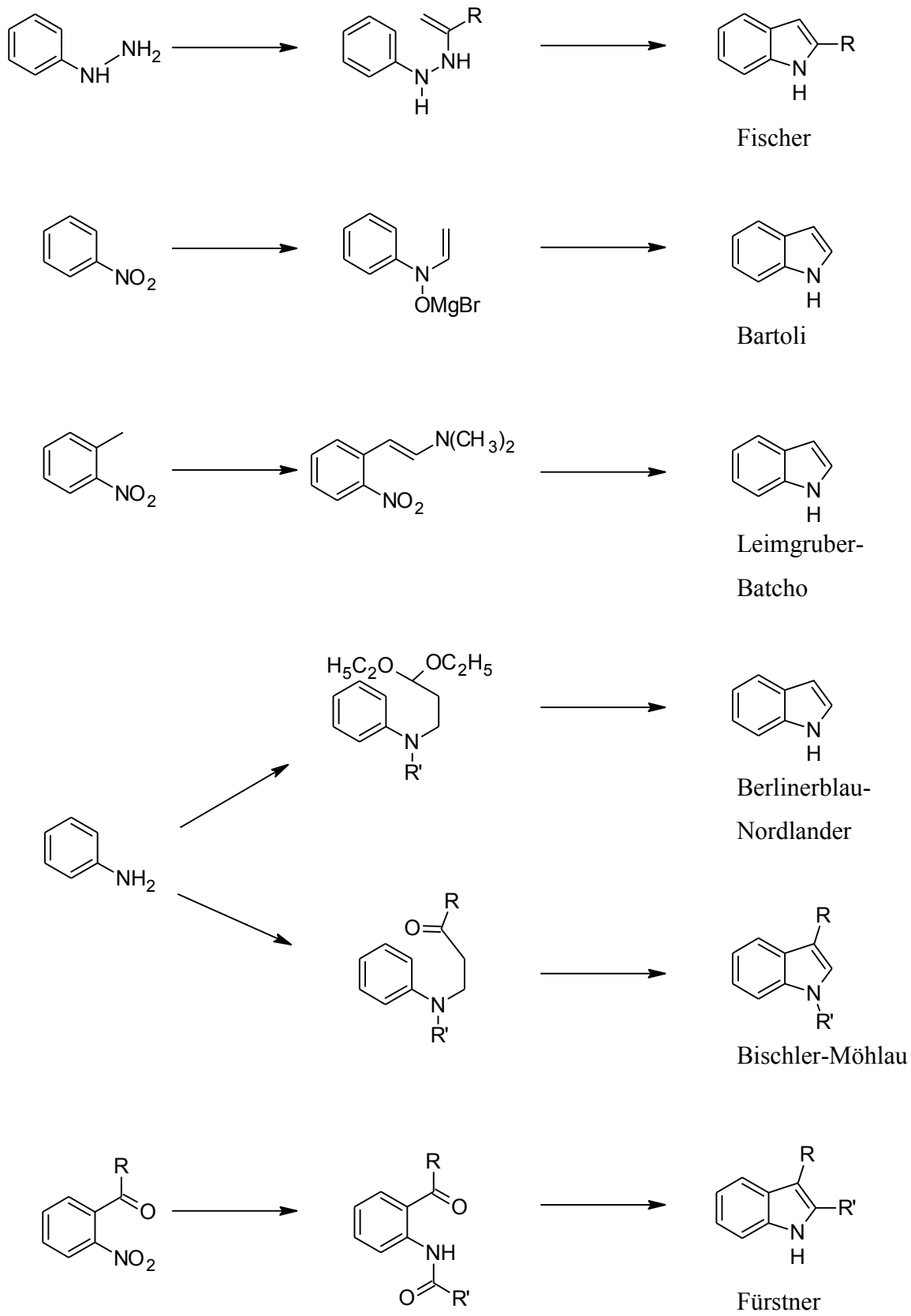
1.3.1 Indole chemistry

Due to the arising interest in indoles and indole chemistry, many attempts have been made to synthesize indoles and derivatives thereof. The first organic synthesis of indole was achieved by Baeyer et al. who oxidized indigo to isatin, reduced isatin to dioxindole and oxindole with zinc dust and further reduced the formed oxindole and dioxindole to indole by passing its vapor over hot zinc oxide (Scheme 15).



Scheme 15 Synthesis of indole performed by Baeyer et al.[72]

Until now a variety of methods has been described for the synthesis of the indole moiety usually classified by the name of their inventors (Scheme 16).

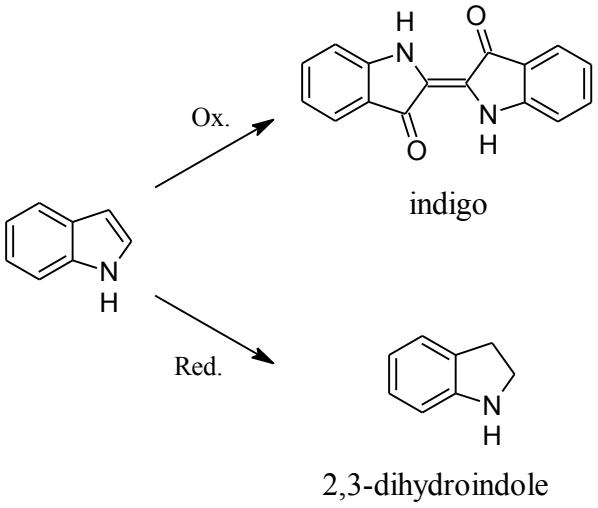


Scheme 16 Selection of pathways for the synthesis of indoles.

The most popular herein is probably the Fischer indole synthesis which uses arylhydrazines and ketones. Here, two new bonds, the N–C^{α} and the C^{β}–C^{ortho} linkage, are formed in the same step. This is equally the case with the Bartoli-Reaction in course of which vinylmagnesium bromide adds nucleophilically to a nitrosoarene, generated in situ from a nitroarene precursor. [73]

The Leimgruber-Batcho synthesis also starts with a nitroarene but herein a methyl group is attached in *ortho* position to the nitro group.[74] Due to the availability of anilines they were also often used for the formation of indoles. Two examples for this are the Berlinerblau-Nordlander[75],[76],[77],[78],[79] and the Bischler-Möhlau[80] procedure. By both methods the aniline is alkylated with a side chain that contains a protected or unprotected β-oxo group which allows an intramolecular Friedel-Crafts hydroxylation by an acid catalyst. Further Fürstner et al. demonstrated that an *ortho* and doubly *N*-acylated aniline also delivers an indole when treated with McMurry low-valent titanium.[81],[82]

The chemistry of indoles is rather versatile. Usually indigo is formed when indoles are subject to mild oxidation conditions such as to hydrogen peroxide or ozone. Reduction of indoles usually leads to 2,3-dihydroindole and is possible under electrolytic conditions or by catalytic hydration. (Scheme 17).[70]



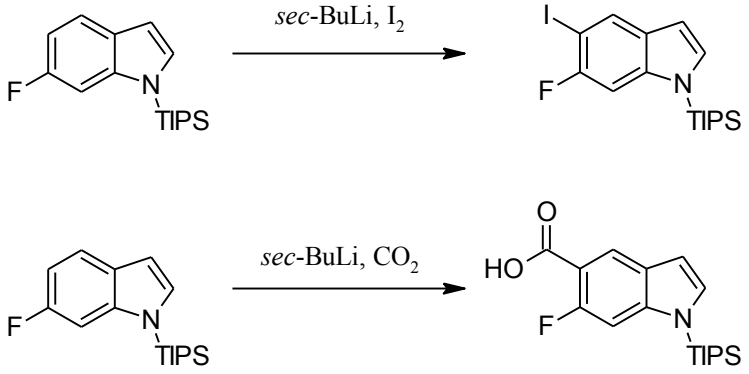
Scheme 17 Reactions of indole under oxidative and reductive conditions.

Furthermore, indole is highly activated in its 3-position towards electrophilic substitution reactions such as nitrations or formylations. One popular example for an electrophilic

substitution reaction that provides formylation in the 3-position is the Vilsmeier-Haack reaction [83] which gives high yields.[84]

In the past, direct halogenation of the carbocycle was not feasible. This was usually done *via* build-up syntheses such as the Bartoli reaction starting from halogenated benzene derivatives. In contrast halogenations in the 2- or 3-position proceed smoothly. Thereby the choice of the reaction conditions and the substitution pattern of the indole determine whether the 2- or the 3-position is halogenated.[85] This is similar with its alkylation which can also occur in the 3-position or at the nitrogen atom depending on the reaction conditions.[86]

Nowadays halogenation and electrophilic aromatic substitution at the carbocycle became possible due to the application of special conditions and reagents such as those described by Schlosser et al. (Scheme 18). [69]



Scheme 18 Electrophilic iodination of 1-TIPS-6-fluoroindole with *sec*-BuLi and I_2 as electrophile (top) and carboxylation of 1-TIPS-6-fluoroindole with *sec*-BuLi and CO_2 as electrophile (bottom).[69]

1.4 α-Amino acids

α-Amino acids are important organic compounds that are biologically active and necessary for a broad variety of biological processes, especially as building blocks for proteins. Every amino acid contains, as described by their name, an amino group and a carboxylic acid. The amino acids involved in biological processes are all α-amino acids. Those amino acids have a central carbon atom, the α-carbon, to which are attached the amino group, the carboxylic acid, a proton and a residue (R), which is often called side chain (see Figure 3). Except of glycine where the side chain is a proton all amino acids are chiral and are therefore D- or L-stereoisomers. The form usually found in naturally occurring proteins is the L-form. [87],[88]

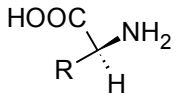


Figure 3 General structure of an α-L-amino acid.

The 20 α-amino acids that are mainly occurring in mammalian proteins are called "standard amino acids" and are divided in *essential* and *non-essential* amino acids. The *essential* α-amino acids cannot be synthesized by the human body and must be delivered by food while the *non-essential* amino acids can be synthesized by the human body.[89] Various ways for classification of the different types of α-amino acids have been described in the past and can be found in the literature.[88]

Some amino acids function not only for nutrition, as building blocks in protein synthesis and cell division but also as metabolic intermediates like in the biosynthesis of neurotransmitters such as L-DOPA or serotonin. The most important of such neurotransmitter amino acids are the ones taking part in the dopaminergic and serotonergic pathways namely phenylalanine, tyrosine and tryptophan.

1.4.1 α-Amino acid uptake through the cell membrane

Generally there are two possibilities for transport of amino acids into cells. They can get into cells by diffusion through a concentration gradient which, however, plays a minor role under physiological conditions. The transport mainly occurs by carrier mediated processes that involve the binding of the substrate to a specific catalytic site on a transporter protein.[90]

Various functionally and biochemically distinct amino acid transporter systems have been discovered and defined on the basis of their amino acid selectivity and physico-chemical properties. In most cases the transport of amino acids into the cytoplasm is an active process that requires binding of the amino acid to an active site on a complex protein that may contain several transmembrane regions. It is well known that most of the carrier proteins transport a special class of amino acids (i.e., neutrals, cationics, aromatics, amino acid amides) rather than one specific amino acid.

Furthermore, many of the transporter systems require sodium for maximal activity and thereby the catalysis of the amino acid transport is coupled to the sodium ion. The transport process itself is established by a high extracellular/intracellular sodium gradient (ca. 140 mM

outside, ca. 10 mM inside). The initial step of the transport is the binding of sodium to the transporter. Thereby a conformational change occurs which increases the affinity of the extracellularly oriented cotransporter site for subsequent amino acid binding. A sodium/amino acid cotransporter complex is formed and another conformational change results in the release of the amino acid and the sodium ion into the cytoplasm inside the cell. The sodium ion is then transported out of the cell via an energy requiring Na^+/K^+ ATPase against its gradient to maintain the electrochemical potential and the sodium gradient of the cell while the released amino acid undergoes intracellular metabolism or functions in signal transduction (Figure 4).[91],[92]

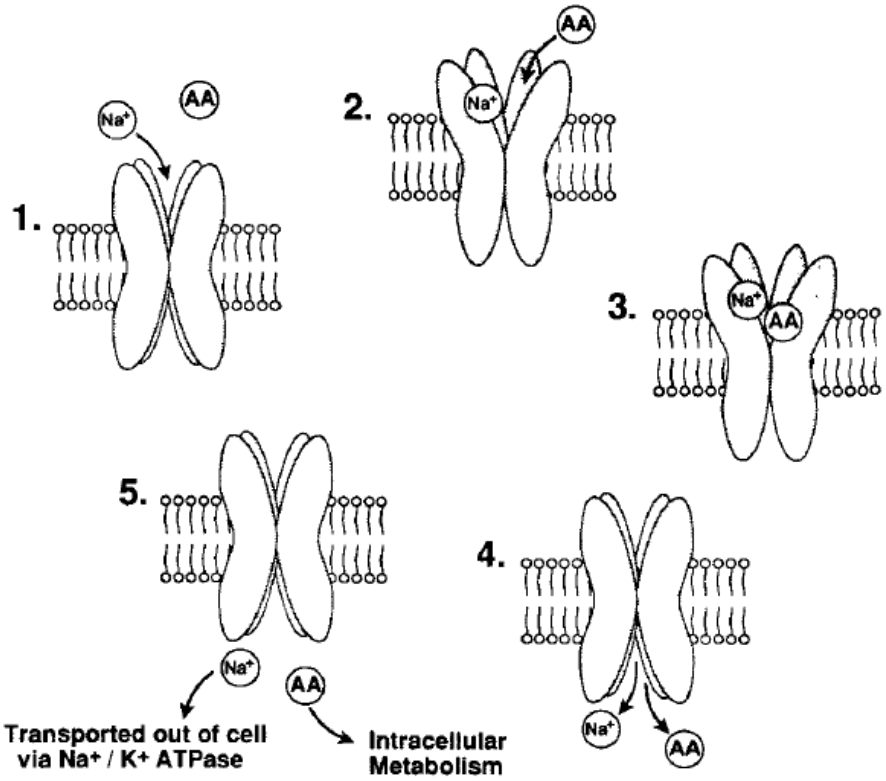


Figure 4 Conceptual scheme of a membrane-bound sodium-dependent α-amino acid carrier.[93]

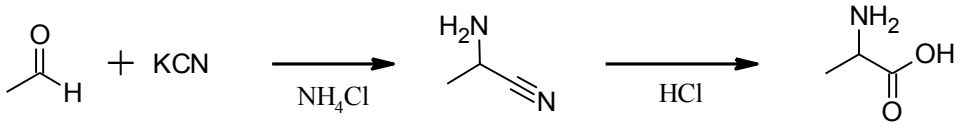
The driving force of this process is the sodium gradient as well as the electrical potential which promotes the initial binding of the sodium ion within the carrier and thereby activates the channel.

For the other group of amino acid transporters – the sodium independent systems – the vectorial movement of amino acid transport generally depends on the relative concentrations of the concerned amino acid inside and outside of the cell and follows the concentration

gradient.[93] There are also examples where the transport occurs against the concentration gradient under appropriate conditions. For example due to it's high "exchange" transport properties, system L, which is responsible for the transport of aromatic amino acids, can accumulate amino acids against their gradient by counter-transporting a second amino acid whose gradient has been established by one or more of the sodium-dependent systems.[94]

1.4.2 Organic synthesis of α-amino acids

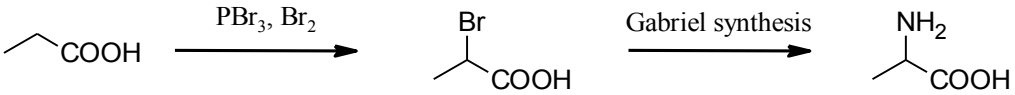
In the past many attempts have been made for the synthesis of different types of α-amino acids. One of the first methods was described by Strecker[95] who first synthesized alanine by reacting acetaldehyde with potassium cyanide and ammonia followed by the addition of an excess of hydrochloric acid (Scheme 19).



Scheme 19 Synthesis of racemic α-amino acids via Strecker's approach.[95]

The reaction itself is a Mannich type reaction and proved useful for the synthesis of several amino acids. The major drawback herein is that the produced amino acids are only available as a racemic mixture.

Another important pathway for the preparation of amino acids is the Hell-Vollhard-Zelinsky reaction in combination with Gabriel's synthesis. Hereby a carboxylic acid is brominated with phosphorous tribromide and elemental bromine followed by a reaction of the resulting α-bromide with potassium phthalimide and subsequent hydrolysis (Scheme 20).[96]



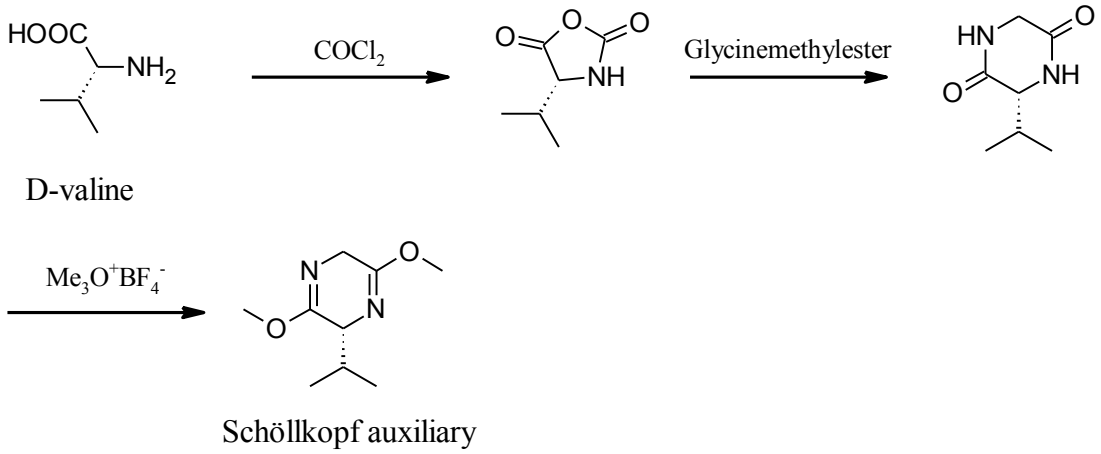
Scheme 20 Synthesis of racemic amino acids via the Gabriel synthesis.[96]

As described with the Strecker method it is not possible to get the desired amino acid in a pure enantiomeric form. Since the separation of two enantiomeric forms usually results in

complicated chiral derivatization into diastereomers followed by separation, procedures are of great advantage, that deliver the desired amino acid with high enantiomeric purity.

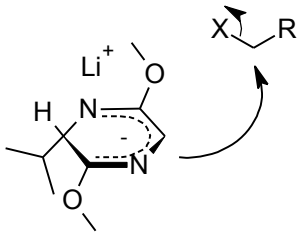
A general method for this purpose is based on an electrophilic alkylation of optically active derivatives of glycine. Two reagents are often used for that and are described in detail below.

One of such glycine derivatives that allows a stereoselective synthesis of amino acids was developed by Schöllkopf and is based on a chiral bislactimether that is derived from glycine and D- or L-valine. The two amino acids are condensed and the carbonyl oxygens are methylated (Scheme 21).



Scheme 21 Preparation of Schöllkopf's auxiliary.[97]

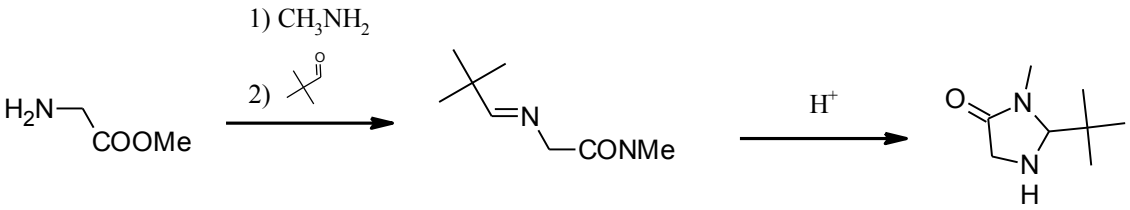
The Schöllkopf's auxiliary is converted into an amino acid by deprotonation and subsequent reaction with an alkyl or aryl halide. Deprotonation of the auxiliary results in a planar transition state (Scheme 22) where an attack of the halide is only possible from the site which is not sterically occupied by the isopropyl residue, resulting in an amino acid with inverted stereochemistry compared to the bislactimether.[97]



Scheme 22 Transition state of the alkylation with Schöllkopf's auxiliary.[97]

Enantiomeric purities obtained using this procedure are generally > 95 %. An improved method was developed using D- or L- *tert*-leucine instead of the naturally occurring form resulting in enantiomeric purities of > 98 %.[98]

Another method relying on a chiral auxiliary is the one developed by Seebach et al.[99] The auxiliary herein is based on an imidazolidinone that is prepared in a multi-step synthesis (Scheme 23). The major drawback is that the auxiliary is obtained in racemic form and needs to be separated by co-crystallisation with L- madelic acid.



Scheme 23 Preparation of Seebach's reagent.[100]

After crystallization the free amine needs to be protected with a Boc- or Z-group. The stereoselectivity results from the bulky *tert*-butyl group that prevents a nucleophilic attack from the site of the imidazolidinone that is occupied by this group. Using this method enantiomeric purities of > 98 % are usually obtained.

1.4.3 Tumor imaging with radiolabeled α-amino acids

Tumor imaging with radiolabeled amino acids is based on the elevated uptake of radiotracer in the malignant tissue.[101] Amino acids are required by all types of cells for energy production, protein synthesis and cell duplication. Malignant tumor cells are known to be most often hypermetabolic in glucose metabolism, protein synthesis and amino acid uptake.[102] Furthermore, the transporter types LAT1 and A1 are often overexpressed in malignant tumor cells as compared to normal tissue.[103],[104],[105],[106] In the literature especially analogues of phenylalanine and tyrosine are described as useful for the imaging of tumors related to overexpression of the LAT1 transporter protein.[107]

Several studies have described the measurement of both protein synthesis rate (PSR) or only the uptake of amino acids by the overexpressed transporters LAT and A1. It is also indicated that in some cases radiolabeled amino acids do have an advantage over FDG which is the standard PET tracer for tumor imaging. This is especially true when it comes to applications

where imaging with FDG is limited. Examples for this are areas of tissue with a high basic glucose uptake such as the brain, or if tumors must be differentiated from inflammatory lesions which is due to high FDG uptake in macrophages, e.g., after radiotherapy or inflammation.

In the past it was suggested by several studies that for tumor imaging it is more important to measure the uptake of amino acids rather than the incorporation into proteins since the latter is slow and does not occur significantly during the time of the measurement. Therefore, it is likely that the fraction of amino acids which is incorporated into proteins does not considerably contribute to the szintigraphic visualization of the tumor.[108],[109],[110]

Subsequently amino acid derivatives that are not subject to protein synthesis but undergo internalization have been developed for the quantification of the amino acid transport.[109],[111],[112],[113] The most prominent of those amino acid analogues is possibly *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine which is widely used for tumor diagnosis.[114],[5],[115] The fact that those slowly metabolised, radiolabeled amino acids are not released from the cells into circulation allows simple kinetic modeling for quantification.

Another major advantage of measuring amino acid uptake rather than protein incorporation is that it is a fast metabolic process and that therefore tumor imaging can be performed within 20 min post injection.

1.5 The serotonergic system

The serotonergic system plays a central role in the regulation of cognitive, emotional and neuroendocrine processes and is related to a broad variety of behavioral functions. Dysfunctions in the signal transduction of the serotonergic system are therefore related to neuropsychiatric diseases as well as to alterations in emotional and cognitive functions.

The serotonergic system is characterized by its broad allocation in the central nervous systems. The neurotransmitter serotonin (5-hydroxytyramine, 5-HT) itself shows a variety of mechanisms of action caused by the interaction with different receptor types and the resulting effects. Serotonin can interact with receptors located pre- and postsynaptic, and according to the subtype of the corresponding receptor, excitatory and inhibitory effects are possible. The main effects of serotonin are the modulation of dopaminergic, cholinergic and GABAergic neurons.[116]

Serotonin was first discovered as a vasoconstrictor substance in the blood[117] and has been revealed to be of importance in a broad range of physiological processes. It has been shown that serotonin is involved in the control of smooth muscle tone and vascular function[118],[119],[120], hemostasis and platelet function[121],[122] and hepatitis and liver regeneration[123],[124],[125],[126]. Further, it was found that it also plays an important role in mammary gland plasticity[127], insulin secretion[128], the development of neurons and sleep regulation. It is also involved in the control of appetite, the gastrointestinal motility, pain sensation, nociception, mood, stress, maternal or sexual behavior and aggression.[129],[130],[131]

The biosynthesis of serotonin starts with the essential amino acid tryptophan which is hydroxylated in 5-position yielding 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase (TPH). This hydroxylation is the initial and rate limiting step[132] in the synthesis of serotonin. 5-HTP is then further decarboxylated (see Scheme 24) to serotonin (5-hydroxytaramine, 5-HT) by aromatic amino acid decarboxylase (AADC). TPH belongs to the family of aromatic amino acid hydroxylases that also includes tyrosine hydroxylase and phenylalanine hydroxylase. All of them are iron (Fe^{2+}) dependent and have similarities in structure and mechanism.[133]

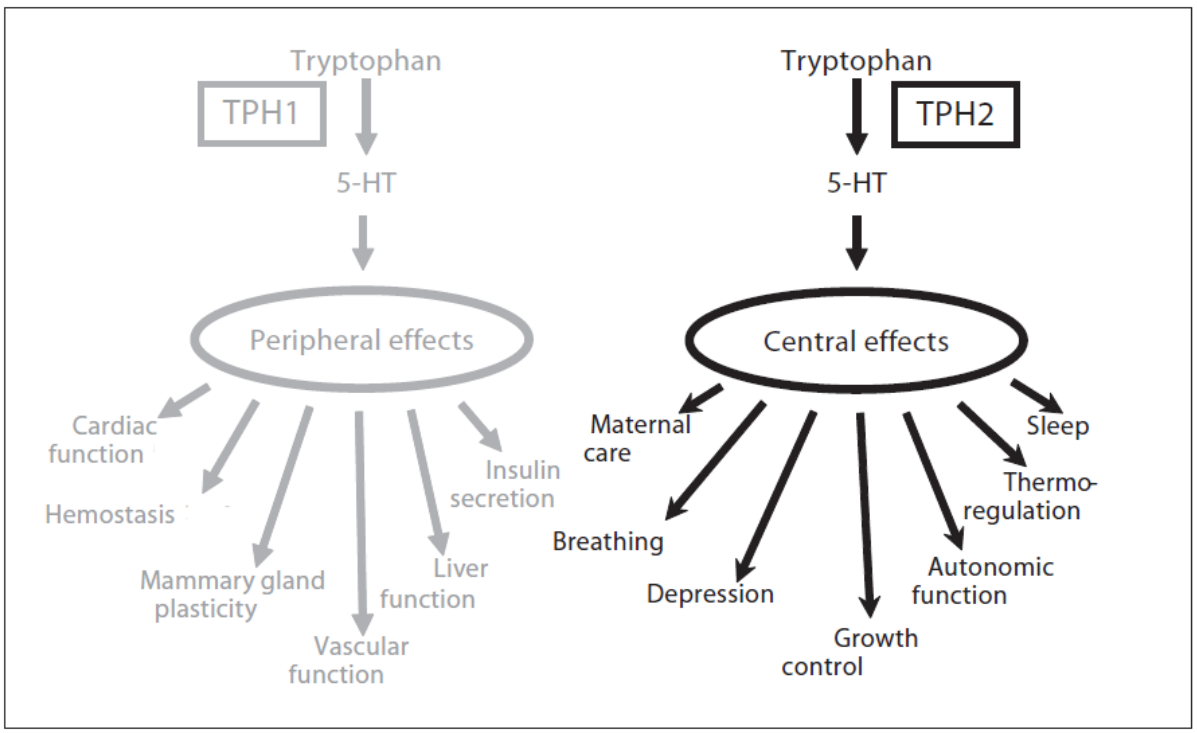
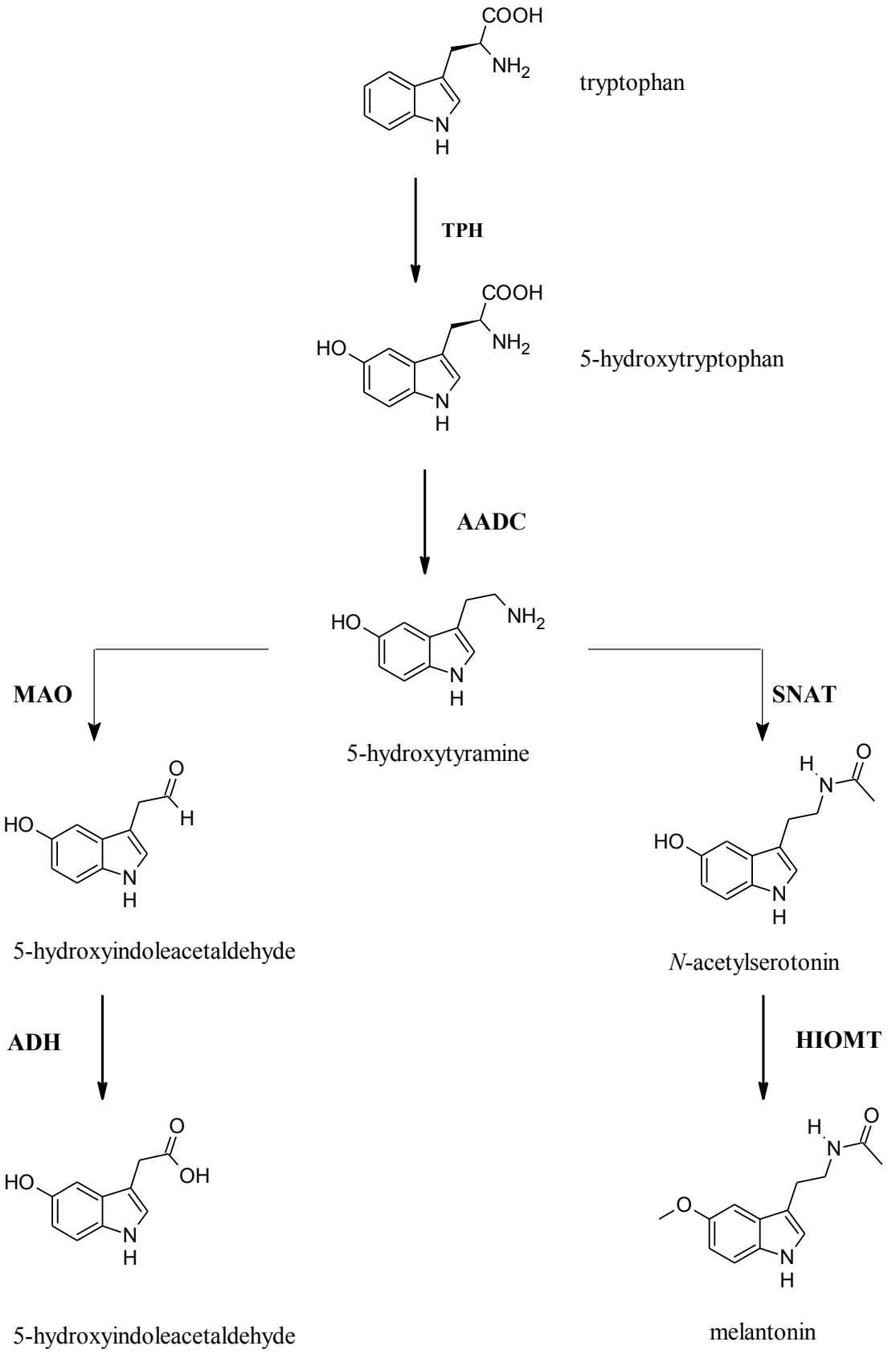


Figure 5 Duality of the 5-HT-system. Functions of peripheral (TPH-1) and central (TPH-2) 5-HT.[134]



Scheme 24 Metabolism of 5-hydroxytryptophan. AADC = aromatic amino acid decarboxylase, SNAT = serotonin *N*-acetyltransferase, HIOMT = hydroxy indole-*O*-methyl transferase, MAO = monoamine oxidase, ADH = alcohol dehydrogenase.[134]

In the past the existence of two independent systems of 5-HT formation has been discovered. The difference of the systems is the rate limiting enzyme TPH. TPH-1 is responsible for the synthesis of 5-HT in peripheral tissue and is found in the gastrointestinal enterochromaffin cells and blood platelets but also in the pineal gland where 5-HT is further metabolized into melatonin. TPH-2 is only expressed in the brain and mainly located in neurons of the raphe nucleus in the brainstem. According to their location both TPH-systems have very different effects on the human body. While TPH-1 has mainly peripheral effects, TPH-2 affects the central nervous system only (Figure 7).

1.5.1 Serotonin receptors

Up to now more than 20 receptors for the neurotransmitter serotonin have been discovered. They are divided into groups from 5-HT_1 to 5-HT_7. The 5-HT_3 receptor is the only ligand-gated ion-channel and belongs to the Cys-loop family of ligand-gated ion-channels such as the nicotinic acetylcholine receptors, the $GABA_A$ receptors, and the glycine receptors.

Table 2 Signaling pathways, location and pharmacology of 5-HT receptor subtypes.[135]

Receptor	Major signaling pathway	Neuronal Location	Regional localization
5-HT_{1A}	↓cAMP	Somatic autoreceptor, postsynaptic	Raphe nuclei/hippo-campus, cortex
5-HT_{1B}	↓cAMP	Terminal autoreceptor, postsynaptic	Striatum, nucleus accumbens, ventral tegmental area
5-HT_{2A}	IP_3	Postsynaptic	Frontal cortex
5-HT_{2c}	IP_3	Postsynaptic	Frontal cortex
5-HT_3	Ion channel	Postsynaptic	Cortex, amygdala
5-HT_4	↑cAMP	Postsynaptic	Striatum, nucleus accumbens, cortex
5-HT_6	↑cAMP	Postsynaptic	Hippocampus, cortex
5-HT_7	↑cAMP	Postsynaptic	Suprachiasmatic nucleus, cortex

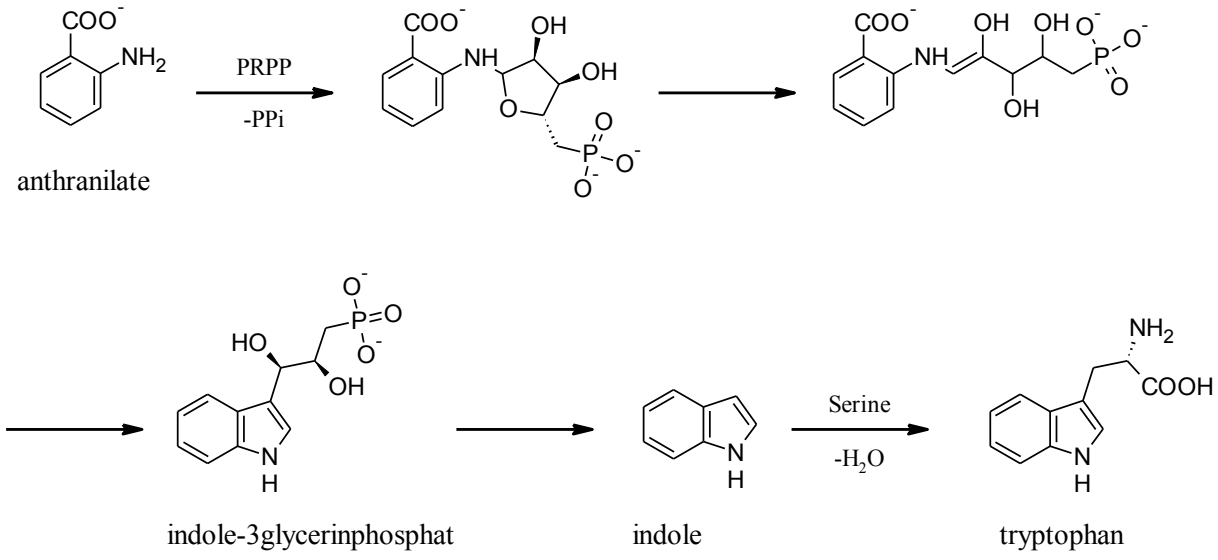
The 5-HT_3 receptors are distributed in both, the peripheral and the central nervous system.[136],[137] The other 5-HT receptors are of metabotropic nature and coupled to G-

proteins. If a single receptor is of excitatory or inhibitory nature, depends on whether it is a presynaptic hetero- or autoreceptor or a postsynaptic receptor. It also depends on the type of second messenger activation the receptor causes. According to the anatomic distribution some receptors are more important for the interaction between serotonin and cognition than others.

The receptors 5-$HT_{1A/1B/1D}$, 5-$HT_{2A/2B/2C}$, 5-$HT_{3A/3B}$, 5-$HT_{4A/4B}$, 5-$HT_{5A/5B}$, 5-HT_6 and 5-HT_7 seem to be highly involved in cognitive processes. They are located in the hippocampus, the basal ganglia, the amygdala, the frontal cortex and partially in other cortical regions.[138] An overview of 5-HT receptor subtypes is given in Table 2.

1.6 Tryptophan

Tryptophan (Trp, W) is one of the essential aromatic amino acids occurring in the human body and belongs to the group of non-polar amino acids. The isolation of tryptophan was first reported in 1901 by Hopkins through the hydrolysis of casein.[139] The biosynthesis of tryptophan in plants and microorganisms usually starts from shikimic acid or anthranilate. The latter condenses with phosphor-ribosylpyrophosphate (PRPP), generating pyrophosphate (PPi) as a by-product. After ring opening of the ribose moiety and following decarboxylation, indole-3-glycerinephosphate is produced which is then transformed into indole. The last step is catalyzed by the enzyme tryptophan synthase. This enzyme couples serine to indole and thereby creates tryptophan (Scheme 25).[140]



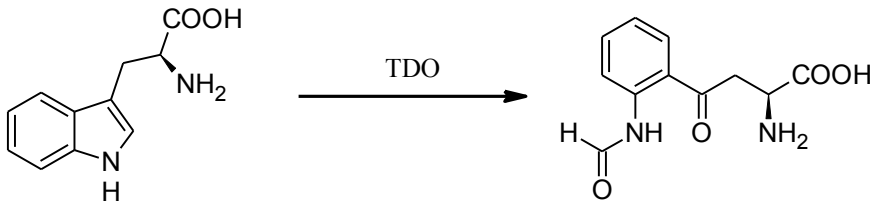
Scheme 25 Biosynthesis of tryptophan starting from anthranilate.

Industrial production of tryptophan also proceeds by the fermentation of indole with serine under the influence of tryptophan synthase. In the last few years tryptophan gained increased interest. It came out that some tumors have a highly elevated tryptophan consumption which could be of use regarding tumor imaging with PET or treatment.[141] Furthermore, tryptophan is the precursor for serotonin which is involved in a broad variety of neurological processes such as depression[134] and migraine[142]. Therefore, a ^{18}F-labeled tryptophan analogue might be a potential radiotracer for imaging of the serotonin synthesis *in vivo*.

1.6.1 Tryptophan in tumor imaging

Recently the assumption came up that fast growing progressive cancers occur because of the failure of the immune system to maintain control over those tumors. Hence, the ability of cancers to escape immune response is attracting attention. There is evidence that the immune escape of those tumors is due to the consumption of tryptophan as a critical factor.[141]

It was found by Opitz et al.[143] that some tumors upregulate the enzyme tryptophan dioxygenase (TDO) to drive tryptophan consumption. This enzyme converts tryptophan to kynurenine (Scheme 26) which is known to be a ligand for the aryl hydrocarbon receptor that mediates invasive tumor growth.[143]



tryptophan kynurenine

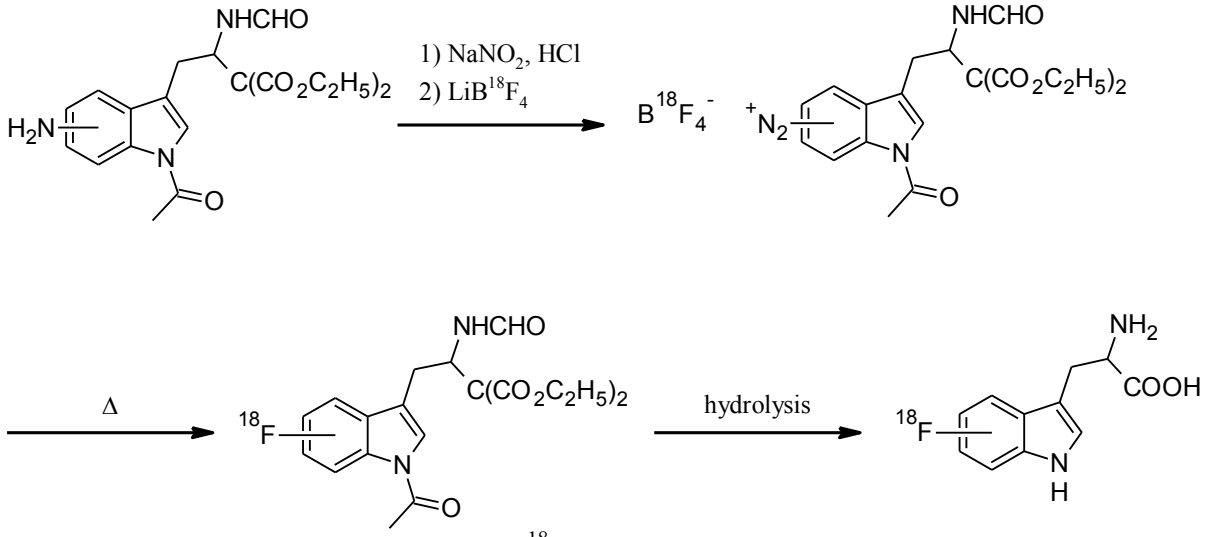
Scheme 26 Conversion of tryptophan to kynurenine.

Those facts may be crucial in the explanation of how tumors overcome immune barriers to cancer progression. It is assumed that the interplay between cancer cells and nearby immune cells plays an important role in whether the cancer is destroyed by the immune system, persists in a dormant, slow growing state or progresses to an aggressive and clinically challenging state. Therefore, immune escape drives the development of tumors towards

aggressive forms.[144] One such pathway is of emerging interest and involves the consumption of tryptophan and the upregulation of the enzymes TDO, IDO (indoleamine 2,3-dioxidase) and IDO2, which are all responsible for the generation of kynurenine through tryptophan metabolism. The crucial role of IDO in immune tolerance was discovered several years before it was connected with cancer. It is also by far the best studied tryptophan metabolizing enzyme.[145],[146] The generation of kynurenine by IDO and TDO inhibits the activation of the so called T cells through various mechanisms which also affect the activity of other immune cells. In patients with cancer the upregulation of IDO is often combined with a poor prognosis.[147] Therefore, radiolabeled tryptophan might be useful for the imaging of cancers with upregulated TDO and IDO.

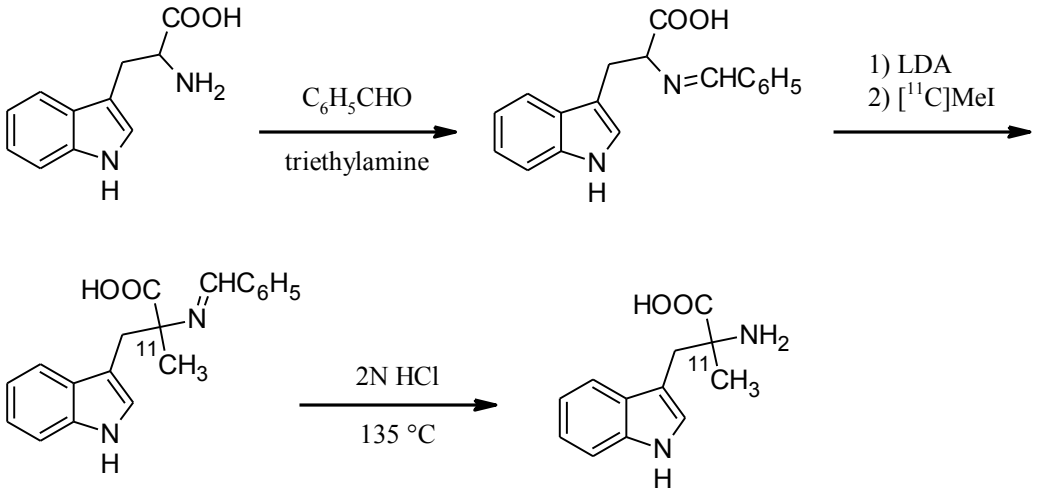
1.6.2 Radiolabeled tryptophan derivatives

In the last 30 years several tryptophan analogues have been radiolabeled with the PET isotopes carbon-11 or fluorine-18. The motivation for this was to find either an amino acid tracer that is suitable for tumor imaging or a tracer that enables the measurement of serotonin levels and metabolism. The first attempt to the radiosynthesis of a radiofluorinated tryptophan analogue was made in 1972 by Atkins et al.[148] who developed a method for 5- and 6-[^{18}F]fluorotryptophan based on a Balz-Schiemann reaction on diethylformamido malonates. The radiosynthesis herein is complicated and time-consuming (>220 min) giving a low RCY between 7 % and 10 % and a specific activity of max. 12.6 μCi/mg. The pathway of this radiosynthesis is shown in Scheme 27.



Scheme 27 Radiosynthesis of 5- and 6-[^{18}F]fluorotryptophan via a Balz-Schiemann reaction.[148]

In 1988 Chaly et al. developed a method for the radiosynthesis of n.c.a. α-[^{11}C]methyl-tryptophan (αMTrp) by alkylation with $^{11}CH_3I$ of an anion which was generated by reacting the Schiff base of L-tryptophan methylester with diisopropylamine (Scheme 28).[149]



α-[^{11}C]methyltryptophan

Scheme 28 Schematic representation of the reaction sequence used in the synthesis of α-[^{11}C]methyltrypytophan.[149]

With this radiotracer the serotonin syntheses rate and the tryptophan uptake from the plasma were determined. The three compartment kinetic model used for the calculation of the k-values is shown in Figure 6. One advantage of this method is, that αMTrp is converted *in situ* into α-methyl-serotonin, a serotonin tracer which was assumed to be distributed into the same compartments as serotonin itself.[150] The experiments were repeated by Muzik et al. who confirmed the results.[151]

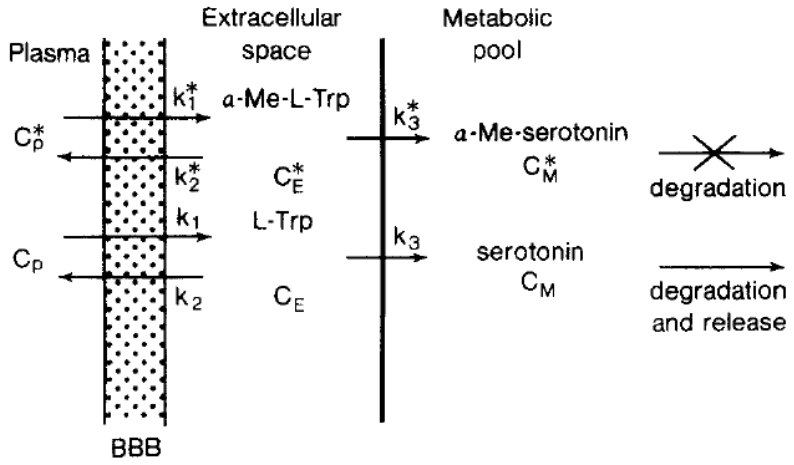
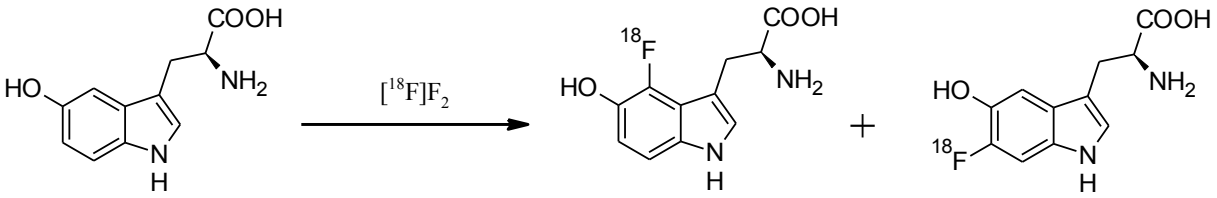


Figure 6 A schematic representation of a biological model representing the metabolic pathway of αMTrp (tracer) and the respective part of tryptophan metabolism.[150]

Later, this was criticized by Gharib et al.[152] and Shoaf et al.[153] who suggested that the trapping of αMTrp is only related to tryptophan incorporation into proteins and thus only an indication of tryptophan uptake into the brain. Further experiments, where specifically one, tryptophan hydroxylase or the protein synthesis, was blocked, recommend that αMTrp is not incorporated into proteins but the uptake is related to serotonin synthesis since it gets metabolized to α-methyl-serotonin.[154]

Also in 1988, a method for the radiosynthesis of [^{18}F]fluoro-5-hydroxy-tryptophan was developed under electrophilic conditions.[155] Herein 5-hydroxytryptophan was directly radiofluorinated with [^{18}F]F_2 resulting in 4-[^{18}F]fluoro-5-hydroxytryptophan and 6-[^{18}F]fluoro-5-hydroxy-tryptophan in RCY of 9 % and 7 %, respectively, and molar activities of about 235 mCi/mmol (Scheme 29). Also a variety of side products was formed that were not further characterized.



Scheme 29 Radiosynthesis of 4- and 6-[^{18}F]fluoro-5-hydroxytryptophan with [^{18}F]F_2.

In 2012 Krämer et al. developed the potential radiotracer 5-(2-[^{18}F]fluoroethoxy)-L-tryptophan (FEHTP) for the purpose of tumor imaging with PET.[156] The radiotracer was prepared by ^{18}F-fluoroethylation starting from [^{18}F]fluoroethyltosylate in a RCY of about 23 %, a specific activity of 50-150 GBq/µmol and a radiochemical purity of > 95 %. The tracer was tested in *in vitro* studies on PC-3, NCI-H69 and MDA-MB-231 cells and showed significantly higher uptake when compared with the uptake of [^{18}F]FDOPA. When *in vivo* metabolism studies were done in xenograft mice, mainly the parent [^{18}F]FEHTP was found in the blood and brain 60 min after injection while [^{18}F]FDOPA could not be detected. The *in vivo* measurements of [^{18}F]FDOPA and [^{18}F]FEHTP showed similar results (Figure 7).

In search of another potential radiotracer based on amino acids, several ^{18}F-labeled fluoropropyltryptophan analogues have been synthesized and evaluated. The tryptophan derivatives investigated were 2-(3-[^{18}F]fluoropropyl)-DL-tryptophan ([^{18}F]2-FPTRP) and 5-(3-[^{18}F]fluoropropyl)-DL-tryptophan ([^{18}F]5-FPTRP). Both compounds were obtained in a RCY of 29-34 % and a radiochemical purity of > 99 %. These tracers are also substrates for

amino acid transport and enter small cell lung cancer cells (NCI-H69) probably via the LAT transporter. *In vivo* PET measurements on xenograft mice showed a high tumor-to-background ratio comparable to that obtained by [^{18}F]FET.[157]

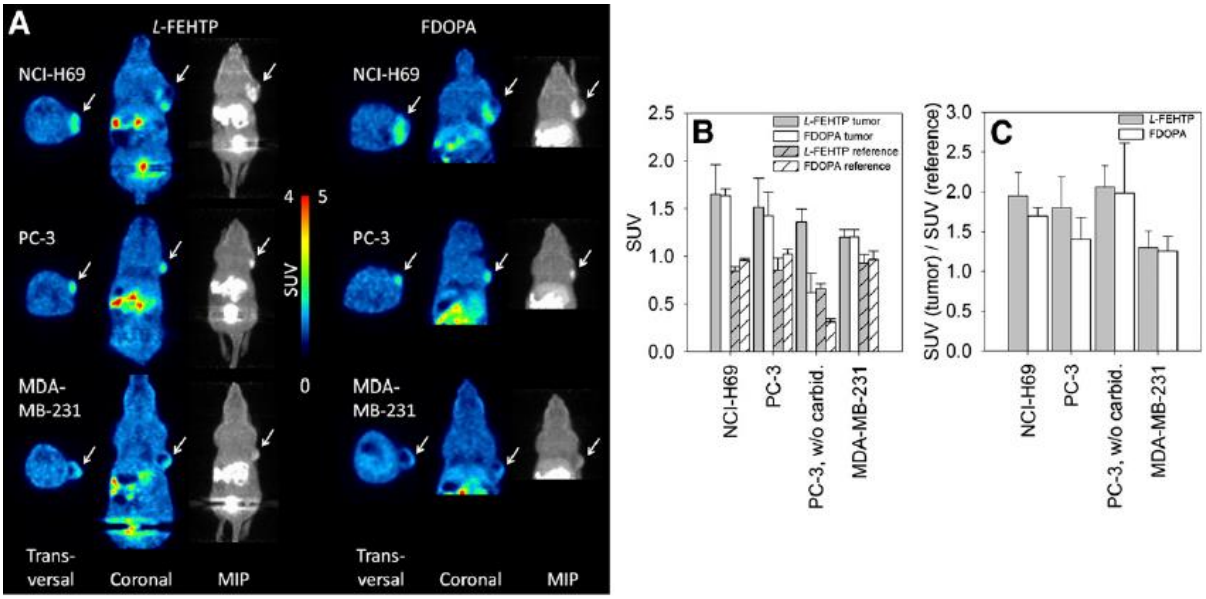


Figure 7 PET analysis of xenograft-bearing mice with [^{18}F]FEHTP and [^{18}F]FDOPA.[156]

2 Aims and scope

^{18}F-labeled amino acids are widely used as radiopharmaceuticals for *in vivo* molecular imaging in tumor diagnostic with PET in nuclear medicine. Up to now procedures for the production of a variety of ^{18}F-labeled amino acids such as 2-[^{18}F]fluoro-L-phenylalanine, 2-[^{18}F]fluoro-L-tyrosine and 6-[^{18}F]fluoro-L-tyrosine are available. Recently the amino acid tryptophan got special interest since there is evidence that many tumors have an increased tryptophan uptake due to overexpression of the enzyme tryptophan dioxygenase (TDO). Furthermore, tryptophan could possibly be used in order to determine biochemical abnormities connected with the serotonergic system, since it is the precursor of serotonin. In the past radiofluorinated tryptophan analogues have only been synthesized by electrophilic fluorination, through secondary groups which alter the original structure, or in a complex and time consuming build-up synthesis with low radiochemical yield and specific activity.

Recently, for the radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine and 2-[^{18}F]fluoro-L-tyrosine, procedures became available with three steps only. Those consist of an isotopic ^{18}F-for-^{19}F exchange, followed by the removal of an activating aldehyde group by reductive decarbonylation, and a final step where the hydrolysis of the protecting groups was done.

Therefore, the major aim of this work was the development of a nucleophilic radiosynthesis which provides L-[^{18}F]fluorotryptophan where the fluorine-18 is attached directly to the carbocycle. This should be accomplished by the three step radiosynthetic concept described above. For this purpose a synthetic pathway was to be developed for the preparation of a suitable, carbonyl activated precursor, in order to realize the radiosynthesis according to the earlier realized concept mentioned above.

In order to determine the optimum positions of the fluorine-substituent and of the formyl group in tryptophan facilitating the isotopic exchange, several *ortho*- and *para*-substituted fluoro-1*H*-indolecarbaldehydes should be prepared with different protecting groups on the indole nitrogen for comparison.

With those compounds the isotopic exchange was to be optimized and to test, if the substitution pattern and the type of protecting group on the indole nitrogen have an influence on the RCY, the chemical stability of the precursor and the labeled compound. Furthermore, a method for a decarbonylation reaction on the fluoro-1*H*-indolecarbyldehydes had to be developed and optimized with respect to RCY.

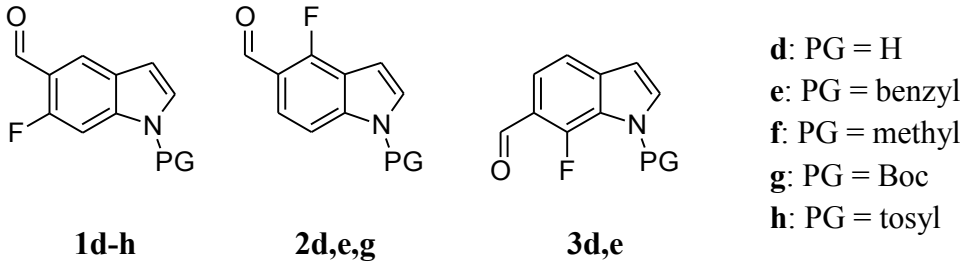
With the experiences on fluoro-1*H*-indolecarbaldehydes, a route for the synthesis of a carbonyl activated precursor should then be developed that allowed an enantioselective radiosynthesis of L-[^{18}F]fluorotryptophan. The positions of the fluorine and the activating carbonyl function herein should be similar to those of the fluro-1*H*-indole-carbyldehyde that gave the best results with respect to RCY and chemical stability.

Subsequently, the radiosynthesis of L-[^{18}F]fluorotryptophan should be realized and each reaction step be optimized on its own. Furthermore, the whole synthesis should be optimized with respect to total time of synthesis and enantiomeric purity of the final product in order to provide a new radiotracer for preclinical evaluation of its usefulness for molecular imaging application with PET.

3 Results and Discussion

3.1 Synthesis of precursors for the radiosynthesis of 1-benzyl-[^{18}F]fluoro-1*H*-indole-carbaldehydes

In recent years the indole moiety has become an important motive in radiopharmacy. Labeling of those compounds with nucleophilic fluorine-18 has only been performed by secondary groups or complex build-up syntheses. Since no literature data exists for the labeling of indoles in the carbocycle, it was attempted to achieve this by an isotopic ^{18}F-for-^{19}F exchange in this dissertation and to study if the substitution pattern has any influence on the RCY and stability of the labeled compounds. In order to reduce the electron density in the benzene ring and thereby activating it for nucleophilic aromatic substitution, an electron withdrawing group was attached in *ortho*- or *para*-position to the fluorine atom. For this purpose several *ortho*- or *para*-substituted fluoro-1*H*-indole-carbaldehydes were synthesized. The structures of the derivatives with *ortho*-substitution pattern are shown in Scheme 30.

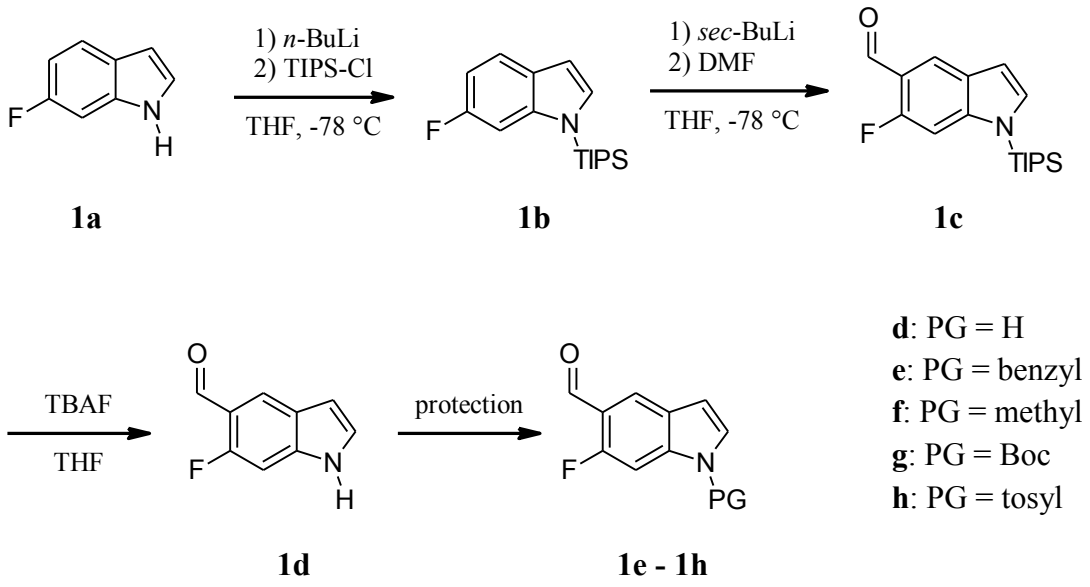


1d-h **2d,e,g** **3d,e**

Scheme 30 Derivatives of indole of interest for isotopic exchange labeling with [^{18}F]fluoride.

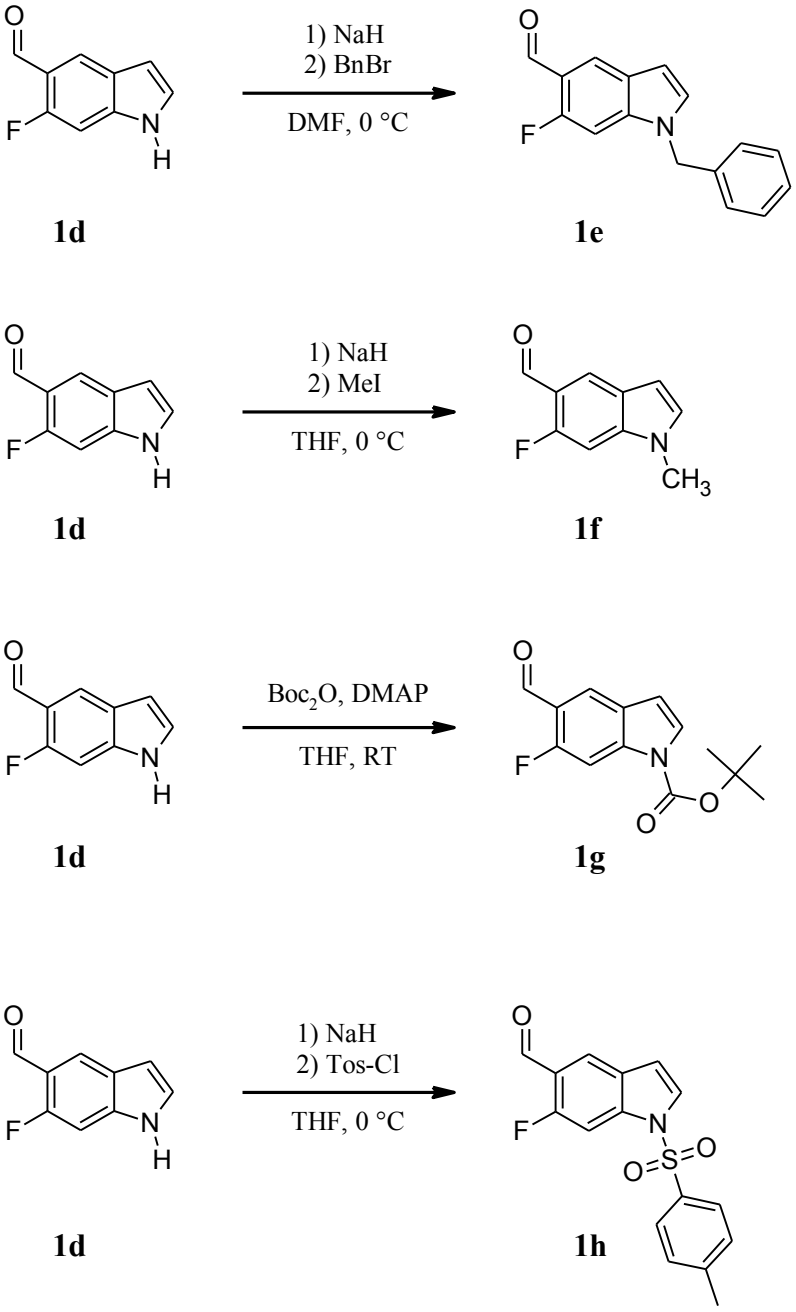
In 2012 Schlosser et al. developed a method for the synthesis of different fluoro-1*H*-indole-carboxylic acids.[69] In this method the critical step was the introduction of the carboxylic acid that was performed by deprotonation of the fluoroindole followed by the addition of carbon dioxide. According to this procedure, a method was developed for the preparation of the *ortho*-substituted fluoro-1*H*-indolecarbaldehydes **1-3**. The route for the whole precursor synthesis is show in Scheme 31 at the example of 6-fluoro-1*H*-indole-5-carbaldehydes (**1d-h**). The first step of the synthesis was the protection of the indole nitrogen which proceeded quantitatively. In this case TIPS (triisopropylsilyl) was the protection group of choice since it is a bulky group that is able to shield the 2-position of the indoles against an electrophilic

attack. Next, the formyl group was introduced *ortho* to the position of fluorine. This was accomplished referring to the procedure described by Schlosser et al.[69] using DMF (*N*,*N*-dimethylformamide) as electrophile instead of carbon dioxide. The formylation gave yields of about 65 % with all fluoro-1*H*-indoles (**1b – 3b)**. Hereby, it was essential to use at least four equivalents of DMF because otherwise much lower yields than 65 % were obtained. The deprotection of the nitrogen was performed with TBAF (tetrabutylammonium fluoride) at room temperature giving yields above 90 %.



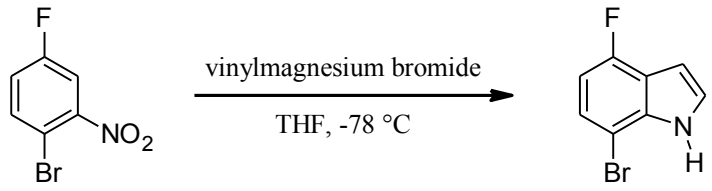
Scheme 31 Synthesis of the *ortho*-substituted fluoro-1*H*-indole-carbaldehyde precursors exemplified by 6-fluoro-1*H*-indole-5-carbaldehyde.

Finally, different protecting groups were attached to the indole nitrogen of the fluoro-1*H*-indolecarbaldehydes. For this the benzyl, methyl, Boc and tosyl groups were used. Scheme 32 shows the protection reactions of all *ortho*-substituted fluoro-1*H*-indolecarbaldehydes (**1d-3d)** at the example of 6-fluoro-1*H*-indole-5-carbaldehyde (**1d**). Except for the *N*-benzylation all protections were performed as described by literature methods.[158],[159],[160] According to the literature, *N*-benzylations of indoles proceed in good yields when THF or DMSO are used as solvent. But for the benzylation of all indole compounds described here (**1d – 3d**) the use of DMF as solvent was essential because otherwise decomposition of the final product was observed. With DMF as solvent the benzylation proceeded in good yields of about 85 %. The methylation gave moderate yields of about 58 % while the tosylation and Boc protection gave yields between 80 and 90 %.



Scheme 32 Protection of the fluoro-1*H*-indole-carbaldehydes exemplified by 6-fluoro-1*H*-indole-5-carbaldehyde.

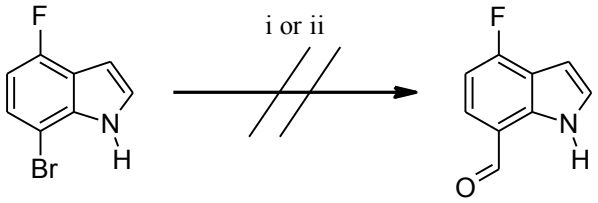
The only fluoro-1*H*-indole-carbaldehyde used with a *para*-substitution pattern was 1-benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (**4e**). An important intermediate for its synthesis was 7-bromo-4-fluoro-1*H*-indole (**4b)** that was prepared in 52 % yield *via* a Bartoli[73] reaction (Scheme 33). The starting material 1-bromo-4-fluoro-2-nitrobenzene was synthesized following the procedure described by Smith et al.[161]



4a **4b**

Scheme 33 Preparation of 7-bromo-4-fluoro-1*H*-indole (**4b**) by a Bartoli reaction.

For the synthesis of the final precursor (**4e**) three synthetic approaches were attempted. The first one was based on a concept described by Schlosser et al[69] who used two equivalents *n*-BuLi followed by the addition of carbon dioxide. This route was modified regarding the reagent used for the bromine formyl exchange. Further DMF was used instead of carbon dioxide in order to generate an aldehyde instead of a carboxylic acid.

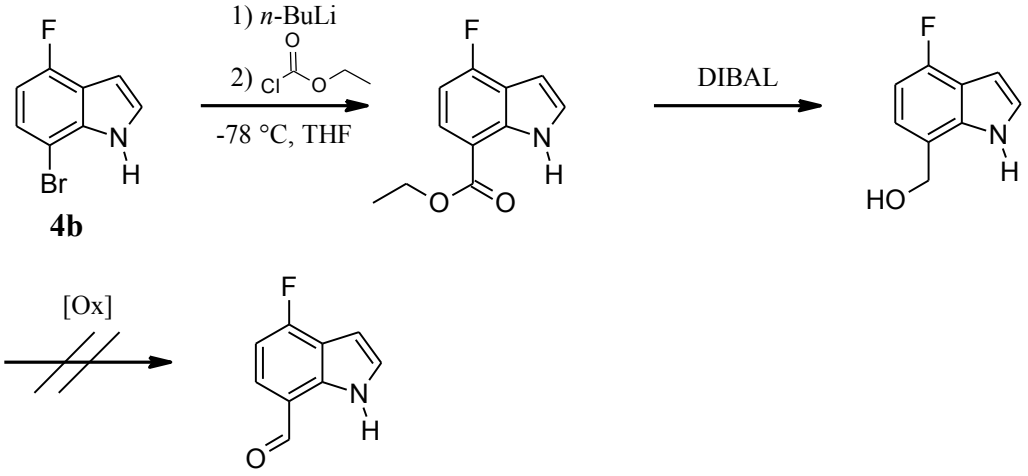


4b **4c**

Scheme 34 Synthesis of 4-fluoro-1*H*-indole-7-carbaldehyde;
i) *i*-PrMgCl.LiCl, THF, 0 °C.; ii) 2 eq. *n*-BuLi, THF, -78 °C.

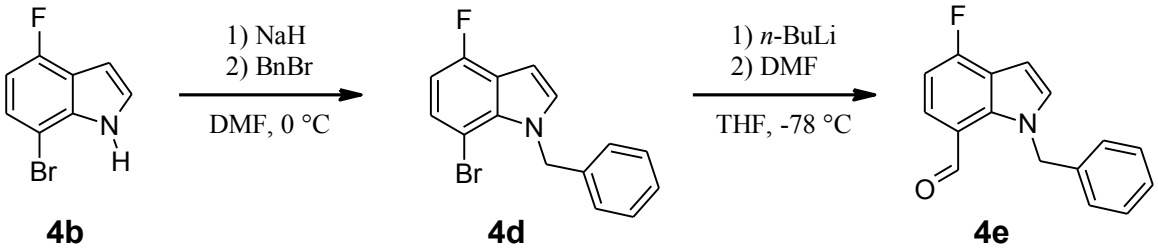
First, *i*-PrMgCl.LiCl was tested as reagent for the bromine-metal exchange, which has been used for similar reactions by Krasovskiy et al.[162]. In contrast to *n*-BuLi, those reagents are known to give high yields and selectivity under very mild conditions. This reaction was performed in THF starting at 0 °C and slowly warming to room temperature after the addition of DMF. Unfortunately, nothing of the desired compound **4c** could be obtained when these conditions were applied. Even when the more active *n*-BuLi was used for the bromine-metal-exchange, it was impossible to get any of the formylated fluoroindole **4c**. But, however, with *n*-BuLi, the debrominated 4-fluorindole could be isolated which is an indication that the bromine-metal-exchange was successful but the following electrophilic substitution did not proceed. Hence, it was assumed that a stronger electrophile than DMF might be more suitable for this purpose. For this reason ethyl chloroformate was chosen as electrophile to give the corresponding benzyl ester. This ester should then be reduced to the alcohol and reoxidized to the aldehyde **4c** (Scheme 35).

Indeed, the esterification and reduction proceeded in good yields of 67 % and 59 %, respectively. The oxidation was tested under various conditions. In the past 2-iodoxybenzoic acid (IBX) has been widely used for mild organic oxidation reactions[163] including the oxidation of benzylic alcohols to benzaldehydes.[164] However, the reoxidation of the benzyl alcohol to the corresponding aldehyde **4c** was not successful with IBX. Instead of the desired indolecarbaldehyde **4c** a variety of side products were found which are probably subject to the formation of di- and oligoindoles. This behavior is described in the literature when some tosyl protected indoles were reacted under oxidizing conditions.[165] Furthermore, the Swern oxidation was tested under standard conditions,[166] but the results were similarly negative compared to the reaction with IBX.



Scheme 35 Attempted synthesis of 4-fluoro-1*H*-indole-7-carbaldehyde (**4c**).

Another approach was made using manganese dioxide (MnO_2) as oxidant which is also generally used for the oxidation of benzyl alcohols to their corresponding benzaldehydes and has previously been used for the synthesis of indole-4-carbaldehyde.[167] However, this attempt was also unsuccessful. The starting material was not converted but could be recovered quantitatively which is an indication that the oxidation potential of MnO_2 is too low for this purpose. Since all those approaches for the reoxidation failed, the synthesis of 4-fluoro-1*H*-indole-7-carbaldehyde could not be accomplished by this pathway. Therefore, a new synthetic route was developed starting also from 7-bromo-4-fluoro-1*H*-indole (**4b**). Herein the nitrogen of the indole was first protected with a benzyl group followed by formylation by a lithium-bromine exchange (Scheme 36).



4b **4d** **4e**

Scheme 36 Synthesis of 1-benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (**4e**) starting from 7-bromo-4-fluoro-1*H*-indole (**4b**).

The benzylation of 7-bromo-4-fluoro-1*H*-indole with NaH and benzyl bromide gave the protected indole **4d** in a yield of 81 %. The following formylation was realized with *n*-BuLi and DMF giving 39 % of the desired 1-benzyl-4-fluoro-1*H*-inole-7-carbaldehyde (**4e**).

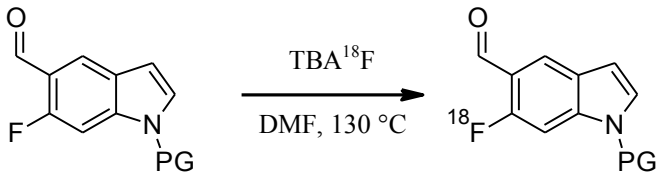
In summary, ten *ortho*-fluoro-1*H*-indole-carbaldehydes (**1d**, **1e**, **1f**, **1g**, **1h**, **2d**, **2e**, **2g**, **3d**, **3e**) with three different substitution patterns and one *para*-fluoro-1*H*-indole-carbaldehyde (**4e**) were successfully prepared and could now be examined for their properties on an isotopic exchange reaction. The *ortho*-fluoro-1*H*-indole-carbaldehydes were obtained in overall yields of 33 – 48 %, depending on the protecting groups that is attached to the indole nitrogen, while the synthesis of the *para*-fluoro-1*H*-indole-carbaldehyde **4e** gave an overall yield of only 16 % which is due to the moderate yield of the Bartoli reaction.

3.2 Radiosynthesis of [^{18}F]fluoro-1*H*-indole-carbaldehydes

3.2.1 Isotopic exchange

The first step in the radiosynthesis of the indolecarbaldehydes was an isotopic ^{18}F-for-^{19}F exchange. Since no data about this type of reaction on indoles exists in the literature the reactions were performed according to data from the literature on isotopic exchange reactions on fluorobenzaldehydes which were assumed to show a similar behavior. Previous work on this nucleophilic aromatic substitution has shown that DMF as solvent, tetrabutylammonium bicarbonate ($TBAHCO_3$) as anion activator and temperatures above 100 °C gave the best RCY.[168],[169]

The labeling reaction was first performed on the 6-fluoro-1*H*-indole-5-carbaldehydes (**1d-h**) where the influence of different protecting groups on the isotopic exchange reaction was examined (Scheme 37).



1d-h **[^{18}F]1d-h**

d: PG = H
e: PG = benzyl
f: PG = methyl
g: PG = Boc
h: PG = tosyl

Scheme 37 Isotopic exchange on 6-fluoro-1*H*-indole-5-carbaldehydes (**1d-h**).

According to the results of the previous works on isotopic exchange reactions on benzaldehyde[168],[169] the reaction was first done in DMF at 130 °C for 15 min with $TBAHCO_3$ as anion activator. These reaction conditions were applied to the unprotected fluoroindole **1d** and to the protected derivatives **1e**-**1h**. The labeling results are shown in Table 3. Unfortunately, the Boc protected compound **1g** which could be deprotected under mild conditions gave only a low RCY of ca. 6 %. Radiofluorination was impossible when the indole nitrogen was not protected or when it was protected with a tosyl group, which was not surprising since detosylation of indoles is commonly carried out with TBAF.[170] The best results could be obtained with the benzyl and methyl protected 6-fluoro-1*H*-indole-5-carbaldehydes **1e** and **1f** where a RCY of about 25 % and 20 % was obtained, respectively. For further experiments the benzyl derivative was used since it gave a slightly higher RCY.

Table 3 Dependence of the radiochemical yield on different protecting groups.

Compound	Protecting group	RCY [%]
1d	none	0
1e	benzyl	25 ± 7
1f	methyl	20 ± 4
1g	Boc	6 ± 2
1h	tosyl	0

Next, the temperature dependence and the influence of the substitution pattern of the benzyl protected fluoro-1*H*-indole-carbaldehydes **1e**, **2e**, **3e** and **4e** on the RCY were investigated. For this purpose the isotopic exchange reactions were done at temperatures between 60 and 150 °C for 15 min. The results are shown in Figure 8.

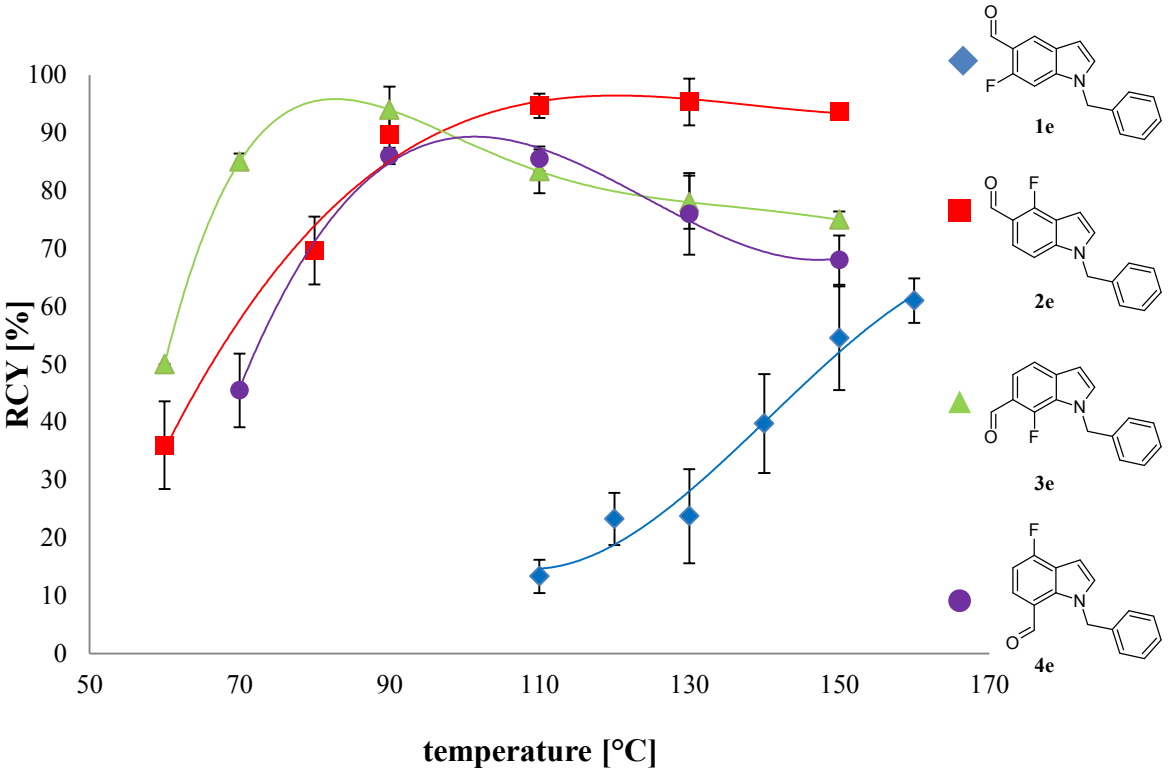


Figure 8 Temperature dependence of the substitution pattern on the isotopic exchange on compounds **1e**, **2e**, **3e** and **4e**.

Surprisingly, it showed that the substitution pattern of the *ortho*- and *para*-substituted indoles (**1e**, **2e**, **3e**, **4e**) has a major impact on the optimum temperature for the isotopic exchange, the maximum RCY achievable and also the chemical stability.

When the isotopic exchange was performed with 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**) at 130 °C a moderate RCY of about 24 % was obtained. With increasing temperatures the gained RCY was also slowly increasing. The maximum RCY was found at 160 °C where 61 % of the labeled compound could be achieved while the formation of any side products was not observed, even at 160 °C. In order to apply higher temperatures than 160 °C, DMSO was used as solvent. However, with DMSO, the highest RCY obtained was 8 % and the formation of several side products was observed at any temperature tested.

Due to the results obtained with 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**), the temperature was first set to 150 °C for 1-benzyl-4-fluoro-1*H*-indole-5-carbaldehyde (**2e**) which resulted in a RCY of about 94 %. Hence, the temperature was decreased until the RCY went down significantly which happened at 80 °C. Between 90 and 150 °C the obtained RCY was always in the area of 90 % or above. Similar to 1-benzyl-6-fluoro-1*H*-indole-5-

carbaldehyde (**1e**), the formation of side products was not observed. Due to these findings it was not possible to determine if the isotopic exchange reaction on 1-benzyl-7-fluoro-1*H*-indole-6-carbaldehyde (**3e**) or 1-benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (**4e**) would rather give results similar to **1e**, **2e** or behave completely different. Therefore, the labeling reaction was investigated at temperatures between 60 and 150 °C for both compounds (**3e** and **4e**). However, the results obtained with **3e** and **4e** were more similar to **2e** than to **1e**. For both compounds (**3e** and **4e**) the maximum RCY that was achieved was around 90 % even though the *ortho*-substituted **3e** gave slightly higher RCY than the *para*-substituted **4e**. The optimum temperature for both compounds was found at about 90 °C. However, decomposition of the labeled product was observed for 1-benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (**4e**) as well as for 1-benzyl-7-fluoro-1*H*-indole-6-carbaldehyde (**3e**) at temperatures higher than 100 °C, which has not been observed for 1-benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (**2e**). The isotopic exchange reaction on the 1-benzyl-fluoro-1*H*-indolecarbaldehyes **2e**, **3e** and **4e** was also done in DMSO and MeCN, but both solvents proved as not suitable for this purpose since low maximum RCY of 8 and 10 %, respectively, were obtained for all compounds along with a variety of side products.

It can be summarized that the substitution pattern has an influence on the properties of the corresponding isotopic exchange reactions. However, not only the position of the fluorine has an influence on the isotopic exchange but also the position of the formyl groups which is significantly exemplified by different results found with the compounds **2e** and **4e**, which both have the fluorine attached in the 4-position and the compounds **1e** and **2e**, that both had the formyl group in the 5-position.

In order to improve the process of the isotopic exchange, a microwave assisted setup was tested on the compounds **1e** and **2e**. It should be examined whether an improvement of the RCY of **1e** and/or a reduction of the reaction time can be obtained. Microwave irradiation produces efficient internal heating (in-core volumetric heating) by direct coupling of microwave energy with the molecules (solvents, reagents, catalysts) that are present in the reaction mixture resulting in an inverted temperature gradient compared to conventional thermal heating.[171] For this purpose the fluoroindoles **1e** and **2e,** $TBAHCO_3$ and dry [^{18}F]fluoride in DMF were irradiated with 20-140 W microwaves during 1 min or 3x1 min, respectively. It proved that the maximum RCY could not be increased for both compounds, but was at least similar as with conventional heating. However, when the reaction mixtures were irradiated for 3x1 min, the RCY of 1-benzyl-4-fluoro-1*H*-indole-5-carbaldehyde (**2e**) decreased at energies higher than 70 W which was not observed under conventional heating.

This was probably due to decomposition of the labeled compound **2e**. A similar behavior was found for 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**) at energies higher than 110 W. Furthermore, with microwave heating an asymptotical dependence of the RCY on the microwave energies was observed for **1e** while a linear dependence was found with conventional heating, which was probably due to the fact that higher temperatures than 160 °C were not applied with DMF as solvent. In summary, the RCY could not be increased but the reaction time can be reduced to 1 min when the labeling reaction is performed with microwave heating.

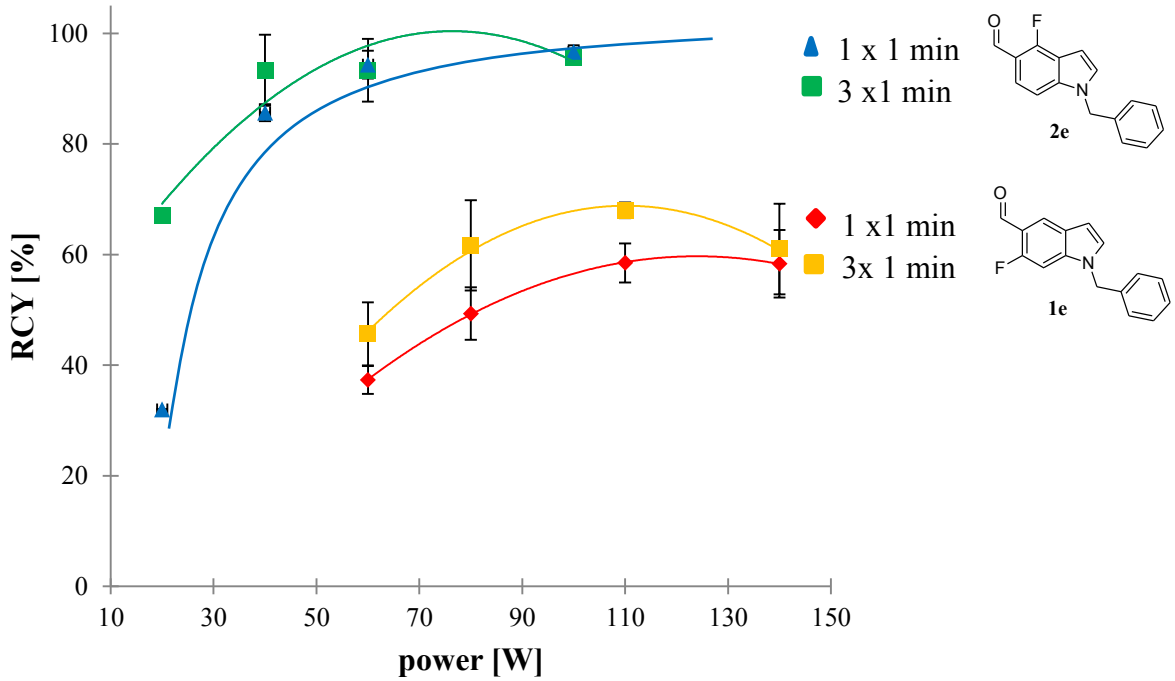


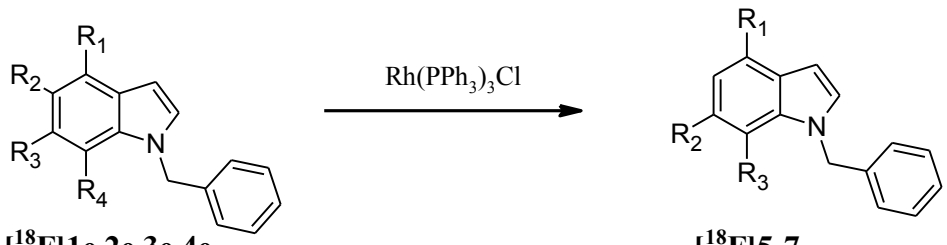
Figure 9 Microwave assisted performance of the isotopic exchange for **1d** and **2d**.

Due to the excellent labeling properties of 1-benzyl-4-fluoro-1*H*-indole-5-carbaldehyde (**2e**) and the opportunity of a mild deprotection, the isotopic exchange reaction was also tested on the Boc-derivative of this compound, 1-Boc-4-fluoro-1*H*-indole-5-carbaldehyde (**2g**). As expected, after optimization of the reaction temperature, a RCY of about 28 % was obtained when the isotopic exchange was performed at 80 °C in DMF with $TBAHCO_3$ as anion activator. At higher temperatures than 100 °C the RCY decreased due to decomposition. However, the Boc-derivative **2g** results in lower RCY than its benzyl derivative, but deprotection of this compound proceeded under mild conditions (2 M HCl, 15 min) in quantitative yields.

In summary, a complex molecule containing the indole moiety should preferably be radiofluorinated in the 4- position, because this position provides outstanding properties with respect to RCY and chemical stability.

3.2.2 Reductive decarbonylation of 1-benzyl-fluoro-1*H*-indole-carbaldehydes

Since the activating aldehyde function is only necessary for the nucleophilic aromatic ^{18}F-for-^{19}F isotopic exchange the possibilities of a removal of this group by a reductive decarbonylation were examined (Scheme 38). This reaction has not been tested on indolecarbaldehydes in the past. For this reason the parameters of previously described decarbonylation reactions on several benzaldehydes with Wilikinson's catalyst ($Rh(PPh_3)_3Cl$) were applied here.[172],[169],[173] Using this reagent for decarbonylation stoichiometric amounts of $Rh(PPh_3)_3Cl$ are required due to the formation of stable $Rh(PPh_3)_3(CO)Cl$ as secondary product.



[^{18}F]1e,2e,3e,4e **[^{18}F]5-7**

[^{18}F]1e: R_1 = H, R_2 = CHO, R_3 = ^{18}F, R_4 = H
[^{18}F]2e: R_1 = ^{18}F, R_2 = CHO, R_3 = H, R_4 = H
[^{18}F]3e: R_1 = H, R_2 = H, R_3 = CHO, R_4 = ^{18}F
[^{18}F]4e: R_1 = ^{18}F, R_2 = H, R_3 = H, R_4 = CHO

[^{18}F]5: R_1 = ^{18}F, R_2 = H, R_3 = H
[^{18}F]6: R_1 = H, R_2 = ^{18}F, R_3 = H
[^{18}F]7: R_1 = H, R_2 = H, R_3 = ^{18}F

Scheme 38 Reductive decarbonylation of 1-benzyl-fluoro-1*H*-indolecarbaldehydes (**1e**, **2e**, **3e** and **4e**) using Wilkinson's catalyst.

Conditions, firstly published in 1992 by Pleneveaux et al.[172], were examined. Dioxane was used as solvent and the reaction was stirred for 20 min at 150 °C in an oil bath. In this setup 2 equivalents of Wilkinson's catalyst were used. With those conditions that gave a good RCY when applied to the benzaldehydes described by Pleneveaux et al. the total conversion of the compound **[^{18}F]1e** was only 29 % while the rest of the starting material remained unchanged. Additionally to the desired product **[^{18}F]6** which was obtained in a RCY of about 6 % the formation of several side products was observed. Due to these disappointing results the reaction was not further optimized with dioxane as solvent.

In 2007 Shen et al.[169] published a method for the decarbonylation of benzaldehydes using benzonitrile as solvent and 3 equivalents of Wilkinson's catalyst. Those conditions were also applied to compound **[^{18}F]1e**. The reaction mixture was again heated to 150 °C for 20 min resulting in a conversion of > 99 %. The desired 1-benzyl-6-[^{18}F]fluoro-1*H*-indole (**[^{18}F]6**) was obtained in an excellent RCY of > 95 %. Applying those conditions to the other 1-benzyl-[^{18}F]fluoro-1*H*-indole-carbaldehydes, **[^{18}F]2e, [^{18}F]3e** and **[^{18}F]4e** resulted in similar RCY, independent of their substitution pattern, which suggests these conditions for the application in decarbonylation reactions on indole compounds.

Furthermore, the decarbonylation reaction was also carried out under microwave heating, based on a method developed by Castillo et al.[173] Herein, benzonitrile was preferred over dioxane as reaction solvent since it gave higher RCY compared to conventional heating as it shows better absorption of microwaves. First, the reaction was tested on **[^{18}F]1e** by irradiation with 100 W microwaves for 50 s using 3 equivalents of Wilkinson's catalyst. This resulted in a RCY of about 85 % of **[^{18}F]6** while no side products could be detected. However, the total conversion was only about 88 % and thereby incomplete. In order to further increase the RCY the reaction time was prolonged to 120 s while the energy of the microwaves was kept at 100 W. This resulted in a conversion of > 99 % and a RCY of about 95 % for **[^{18}F]6**. These results could also be transferred to the compounds **[^{18}F]2e, [^{18}F]3e** and **[^{18}F]4e** giving similar RCY as listed in Table 4. In contrast to the obtained RCY on the isotopic exchange on the fluoroindolecarbaldehydes **1e**, **2e**, **3e** and **4e**, the substitution pattern had no impact on the RCY during the decarbonylation reaction.

The introduction of the microwave assisted setup did not change the RCY or the formation of side products under the optimum reaction time of 2 min. However, it was possible to reduce the reaction time from 20 min to 2 min.

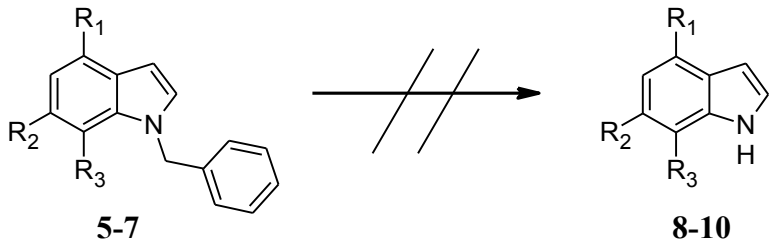
In summary, a reductive decarbonylation on all tested 1-benzyl-4-fluoro-1*H*-indolecarb-aldehydes was successful. When benzonitrile was used as solvent, a RCY of > 95 % was obtained for all compounds under conventional heating as well as under microwave heating. However, the reaction time could be reduced from 20 min to 2 min with microwave heating of 100 W for 2 min.

Table 4 Reductive decarbonylation on 1-benzyl-[^{18}F]fluoro-1*H*-indole-carbaldehydes **[^{18}F]1e**, **[^{18}F]2e**, **[^{18}F]3e** and **[^{18}F]4e** comparing conventional and microwave heating; RCY of the reductive decarbonylation were similar for all compounds and independent of their substitution pattern.

Solvent	eq. Wilkinson	Conditions	Conversion [%]	RCY [%]
benzonitrile	3	150 °C, 20 min [169]	> 99	95 ± 6
benzonitrile	3	100 W, 50 s [173]	85 ± 2	85 ± 2
benzonitrile	3	100 W, 120 s	> 99	95 ± 3
dioxane	2	150 °C, 20 min [172]	29 ± 4	6 ± 3

3.3 Attempts for the debenzylation of 1-benzyl-fluoro-1*H*-indoles

Since most bioactive molecules and pharmaceuticals that contain the indole moiety do not have a protecting group attached to the indole nitrogen and due to the fact that this protecting group provided the best RCY for the isotopic exchange it was examined if it was possible to remove this group during the radiosynthesis (Scheme 39).



5-7 **8-10**

5: R_1 = F, R_2 = H, R_3 = H
6: R_1 = H, R_2 = F, R_3 = H
7: R_1 = H, R_2 = H, R_3 = F

8: R_1 = F, R_2 = H, R_3 = H
9: R_1 = H, R_2 = F, R_3 = H
10: R_1 = H, R_2 = H, R_3 = F

Scheme 39 Attempts for the debenzylation of 1-benzyl-fluoro-1*H*-indoles.

This was first tested with non-radioactive conditions in order to allow more experiments in time. Several previously published methods were investigated for the debenzylation. First the debenzylation was examined with the help of palladium on activated charcoal (Pd/C) in combination with ammonium formate (NH_4HCO_2) in ethanol.[174] Applying these conditions no conversion of the starting material could be detected even when heating the reaction mixture to reflux. Next, a procedure previously described by Petras et al.[175] was studied

where hydrogen gas was passed through a solution of Pd/C in acetic acid, but again no conversion was detected. It has also been described in the literature that the debenzylation of indoles proceeds good with an excess of $AlCl_3$ in aromatic solvents such as benzene, toluene or anisole when the 3-position of the indole is occupied.[176],[177] Although this position was not occupied the procedure was examined. None of the desired debenzylated fluoroindole could be detected but the formation of a variety of side products was observed, probably resulting from the formation of di- or oligoindoles which has previously been observed by Fujino et al. when indoles with an unoccupied 3-position were reacted with $AlCl_3$ under similar conditions.[165] Even if these conditions did not give the desired debenzylated compound, this method is more promising than the ones investigated so far since the 3-position which seems to be the critical part is alkylated in most natural products and pharmaceuticals that contain the indole moiety. Furthermore, a method described by Jorand-Lebrun et al.[178] was examined that was originally used for the debenzylation of tertiary amines such as benzylpiperdidines. Hereby, triphosgene was used to convert the benzylamine into the corresponding carbamoyl chloride which was then hydrolyzed under acidic conditions. This procedure also resulted not in a conversion of the starting material. Also the attempt of an acidic hydrolysis with conc. hydrochloric acid at 150 °C failed, and no conversion was observed. Table 5 summarizes the results obtained for the debenzylation of the different 1-benzyl-fluoro-1*H*-indoles.

Table 5 Approaches for the debenzylation of 1-benzyl-fluoro-1*H*-indoles.

Reagent/Catalyst	Solvent	Temperature [°C]	Conversion [%]	Yield [%]
Pd/C, NH_4HCO_2	EtOH	RT	0	0
Pd/C, NH_4HCO_2	EtOH	110 °C	0	0
Pd/C, H_2	AcOH	RT	0	0
$AlCl_3$	benzene	RT	>99	0
$AlCl_3$	toluene	RT	>99	0
$AlCl_3$	anisole	RT	>99	0
triphosgene	DCM	RT	0	0
HCl	H_2O	150 °C	0	0

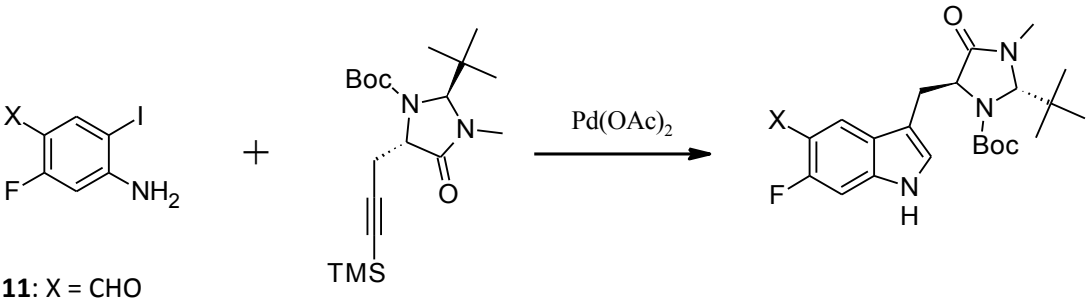
However, a procedure for the successful debenzylation of these 1-benzyl-fluoro-1*H*-indolecarbaldehydes was not found. The approach with $AlCl_3$ is nevertheless promising when indoles are subject to debenzylation where the 3-position is occupied.

3.4 Synthesis of precursors for the radiosynthesis of L-6-[^{18}F]fluorotryptophan

Although the previous results on 1-benzyl-fluoro-1*H*-indole-carbaldehydes have shown that molecules containing the indole moiety should be preferably radiofluorinated in the aromatic ring in the 4-position, a pathway for a precursor could be developed, which is fluorinated in the 6-position , since a promising route for this synthesis was available and previously described by Ma et al.[179] Furthermore, it was shown in this work that indoles carrying a fluorine in this position have proved to be very stable under acidic conditions as well as under basic conditions and are also insensitive towards elevated temperatures which allows a broad variety of reaction conditions during precursor synthesis.

3.4.1 Precursor for the radiosynthesis via a build-up synthesis

The general concept of the route for the synthesis of the precursor is based on a method for the synthesis of tryptophan analogues via a palladium mediated heteroannulation.[179] Herein an *ortho*-iodoaniline is coupled to a functionalized trimethylsilylalkyne. In this case the trimethylsilylalkyne should be functionalized with Seebach's auxiliary in order to generate a stereocenter that enables a stereoselective synthesis of only one enantiomer of 6-[^{18}F]fluorotryptophan. In this case Seebach's auxiliary was preferred over Schöllkopf's auxiliary since it is known to give higher enantiomeric purities. The functionalized alkyne should be coupled to one of the *ortho*-iodoanilines (**11** - **14**). In order to generate the L-enantiomer of tryptophan, the S-enantiomer of Seebach's auxiliary was used for the introduction of the chiral center.

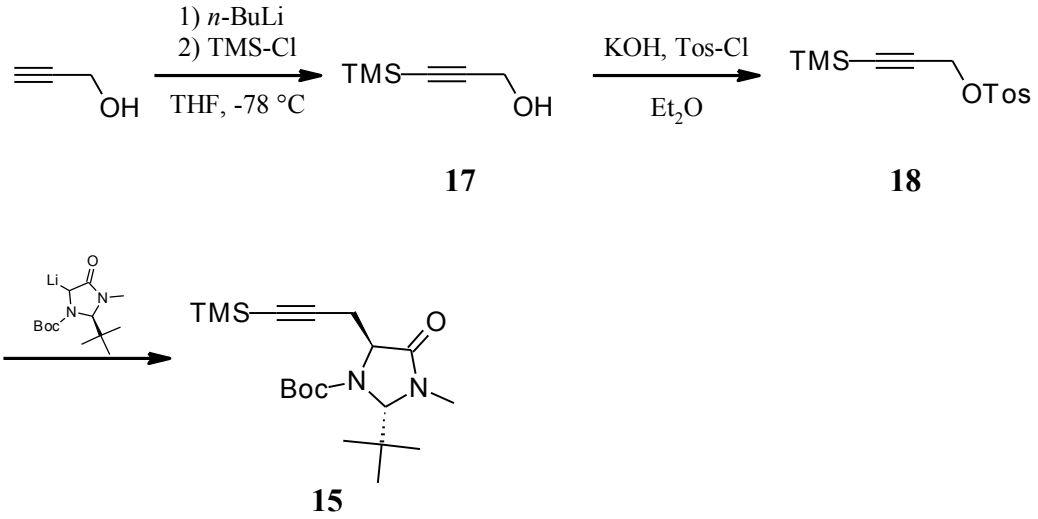


11: X = CHO
12: X = Ac
13: X = Dithiolan
14: X = I

15 **16**

Scheme 40 Synthetic concept for the preparation of a precursor for L-6-[^{18}F]fluorotryptophan.

First, the alkyne **15** was prepared following a modified pathway to the one described earlier by Ma et al.[179] starting from propargyl alcohol. The synthetic route is shown in Scheme 41.



17 **18**

15

Scheme 41 Preparation of Seebach functionalized alkyne **15**.

The first step herein was the introduction of the TMS group. This was accomplished through a deprotonation of both, the alcohol and the alkyne group with *n*-BuLi followed by the addition of two equivalents of TMS-Cl. The TMS group on the alcohol was removed through an acidic work-up yielding the intermediate **17** in 96 % yield. The introduction of the tosyl group was first performed with KOH in Et_2O at room temperature which resulted in varying, rather low yields of 14 - 18 % and a major tosylated but desilylated side product. Therefore, the reaction was examined with triethylamine as base and a reaction temperature of 0 °C giving less than

10 % yield and also the desilylated side product. When the reaction was performed with DMAP (*N,N*-dimethylaminopyridine) as catalyst in DCM, no conversion of the starting material could be observed. Using pyridine as base and solvent at 0 °C resulted in decomposition of **17** while it was impossible to recover any of the starting material. The only procedure that was suitable for this reaction was the use of powdered KOH in Et_2O, starting the reaction at -50 °C and slowly warming it to 0 °C during 2 h. Thereby, the alkyne **18** could be obtained in 87 % yield. Table 6 summarizes the conditions examined for this reaction.

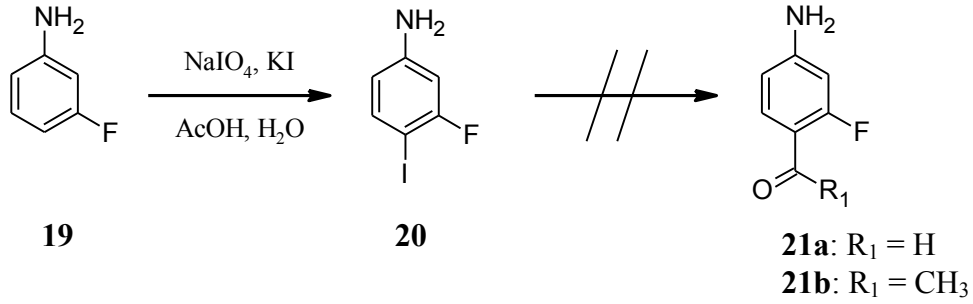
The following coupling to Seebach's auxiliary was performed under standard conditions with LDA (lithium diisopropylamide) as base at -78 °C giving the chiral alkyne **15** in 82 %.

Table 6 Conditions for the tosylation of the alkyne **17**.

Solvent	Base	Temperature	Time	Conversion [%]	Yield [%]
Et_2O	KOH	RT	2 h	>99	14 - 18
Et_2O	Et_3N	0 °C	2 h	>99	<10
DCM	DMAP	RT	24 h	0	0
Pyridine	Pyridine	0 °C – RT	2 h	>99	0
Et_2O	KOH	-50 °C – 0 °C	2 h	>99	87

Next, a pathway for the preparation of a suitable *ortho*-iodoaniline was developed. An overview of the selected routes is shown in Scheme 44. The aim hereby was the preparation of an *ortho*-iodoaniline that contains a fluorine atom as well as an electron withdrawing group such as an aldehyde.

The first approach started with 3-fluoroaniline (**19**) which was iodinated in the 4-position with $NaIO_4$ and KI in aqueous acetic acid[180] giving the desired 3-fluoro-4-iodoaniline (**20**) in 82 % yield. This reaction was carried out at 25 °C for 3 h. Longer reaction times or elevated temperatures promoted the formation of the diiodinated 5-fluoro-2,4-diiodoaniline (**14**) and resulted in decreasing yields for the monoiodinated aniline **20**.



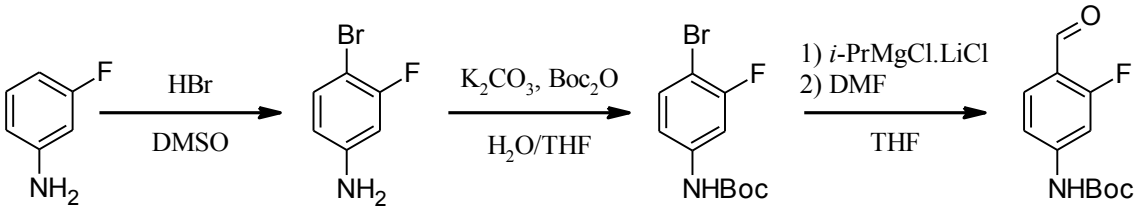
19 **20**

21a: $R_1 = H$
21b: $R_1 = CH_3$

Scheme 42 Synthetic route for the synthesis of 4-amino-2-fluorobenzaldehyde **(21a,b)**.

The iodoaniline **20** should afterwards be converted into the corresponding amino-benzaldehyde **21a**. For this purpose, a method for the formylation of anilines described by Gilman et al.[181] was studied where three equivalents *n*-BuLi were applied to the aromatic amine followed by the addition of DMF at –78 °C. Unfortunately, this reaction led to decomposition of the starting material and none of the desired aminobenzaldehyde **21a** could be isolated. Furthermore, a procedure that has been described by Krasovskiy et al.[162] was examined, which has been applied successfully during the synthesis of the 1-benzyl-fluoro-1*H*-indole-carbaldehydes (see chapter 3.1) and proceeds under milder conditions. Hereby, the reagent for the iodine-metal exchange is *i*-PrMgBr. Equally as under the conditions described above, none of the desired aminobenzaldehyde **21a** could be isolated. Due to the fact that the introduction of the aldehyde function failed, the introduction of an acetyl group was studied. For this purpose the same conditions that have been tried for the formylation of the iodoaniline **20** were applied, but using acetyl chloride as electrophile instead of DMF, since it is known to have a higher electrophilic activity. With this procedure, it was still impossible to isolate the desired aniline **21b**.

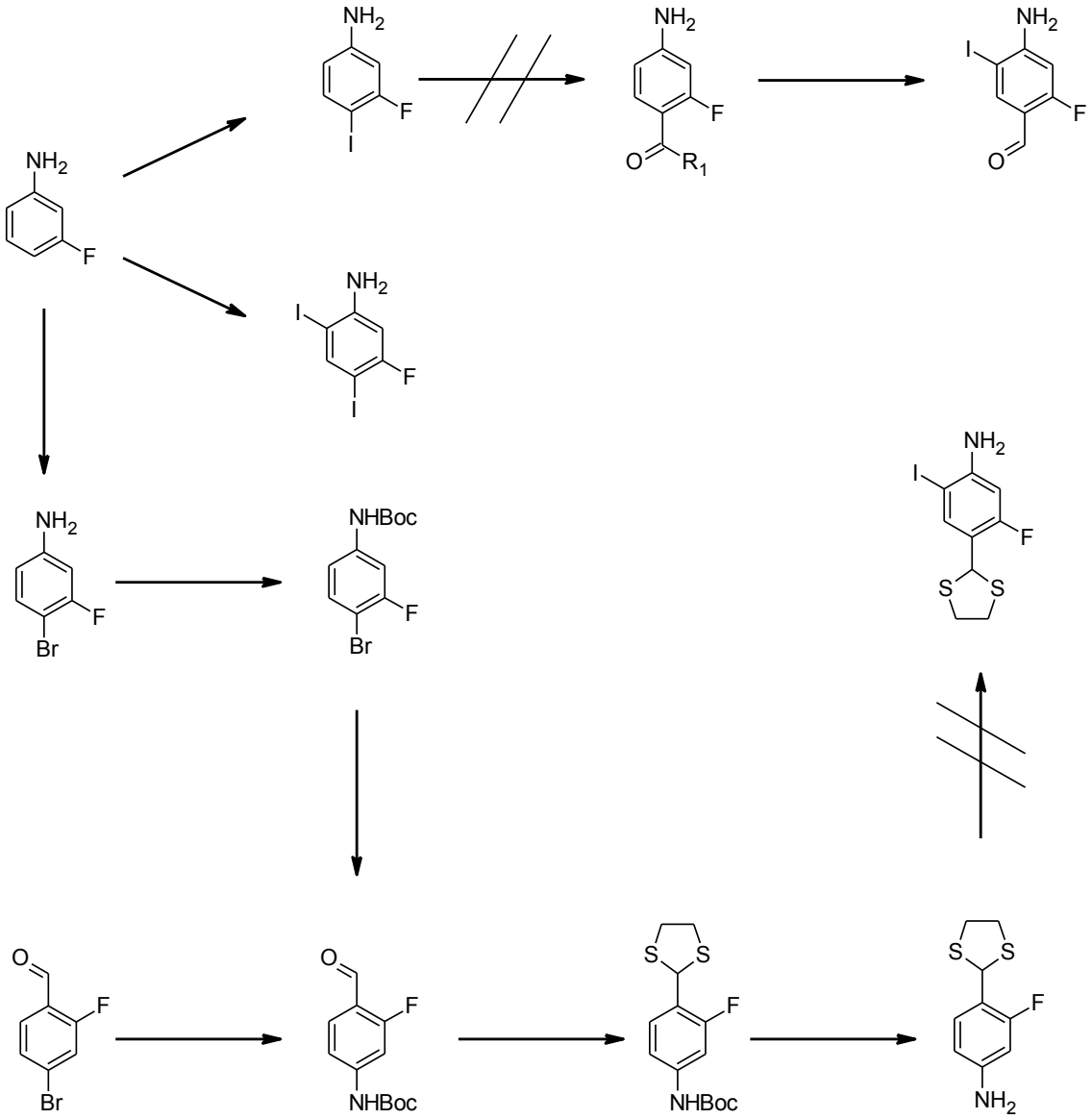
Next, a new pathway starting again from 3-fluoroaniline (**19**) was examined. The first step hereby was the introduction of a bromine *para* to the amino group followed by the protection of the free amine with a Boc-group and a formylation via a halogen-metal exchange (Scheme 43).



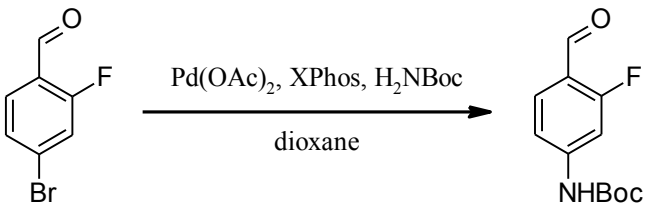
19 **22** **23** **25**

Scheme 43 Preparation of tert-butyl (3-fluoro-4-formylphenyl)carbamate (**25**).

The bromination of the fluoroaniline was done with HBr in DMSO according to a procedure described by Majetich et al.[182] giving the bromoaniline **22** in 82 % yield. The introduction of the Boc-group was done with the help of Boc_2O and K_2CO_3 yielding the bromoaniline **23** in 64 % yield. The bromine-formyl substitution was performed by a halogen-magnesium exchange with the help of *i*-PrMgCl.LiCl at 0 °C following again the procedure described by Krasovskiy et al.[162] and giving the corresponding benzaldehyde **25** in 97 % yield. Furthermore, an improved pathway to this procedure was developed starting from 4-bromo-2-fluorobenzaldehyde (**24**) with the introduction of a Boc-protected amine via a palladium catalyzed heteroamidation (Scheme 45) which was described by Qin et al.[183] giving the protected aminobenzaldehyde **25** in 97 % yield while reducing the number of necessary synthetic steps from three to one (Scheme 45).



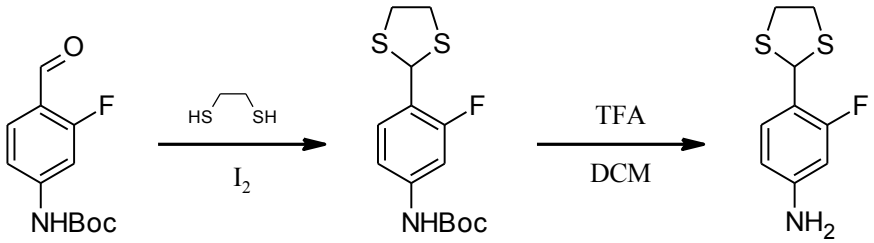
Scheme 44 Overview of the synthetic concepts considered for the preparation of *ortho*-iodoanilines.



24 **25**

Scheme 45 One step synthesis for the preparation of *tert*-butyl (3-fluoro-4-formylphenyl)carbamate (**25**).

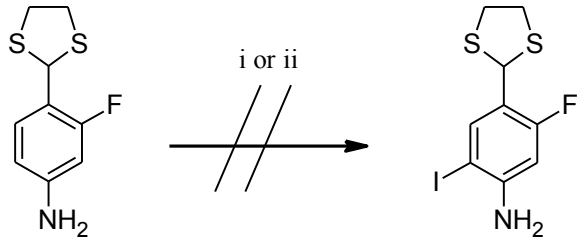
The next steps were the protection of the benzaldehyde **25** and the deprotection of the amine (Scheme 46). For the protection of the benzaldehyde **25** a 1,3-dithiolane group was chosen, since dithiolanes are known to be stable towards a wide variety of reagents and conditions and can be removed with high selectivity under mild conditions.[184],[185] The introduction of the 1,3-dithiolan was performed as previously published by Firouzabadi et al.[186] with 1,2-ethandithiol in DCM and 10 % elemental iodine as catalyst. Using this procedure the dithiolan **26** could be obtained in yields of ca. 90 %.



25 **26** **27**

Scheme 46 Preparation of 4-(1,3-dithiolan-2-yl)-3-fluoroaniline (**27**).

The removal of the Boc group was done under standard conditions with TFA in DCM at room temperature yielding the desired aniline **27** in 77 %. Now, the introduction of the iodine in *ortho*-position to the amine had to be done (Scheme 47). This should be accomplished by the procedure of Emmanuvel et al.[180] which gave good yields when it was applied to 3-fluoroaniline (**19**). With **27**, however, neither the desired *ortho*-iodoanilie **13** nor the starting material **27** could be obtained due to decomposition which was probably caused by the dithiolan group.

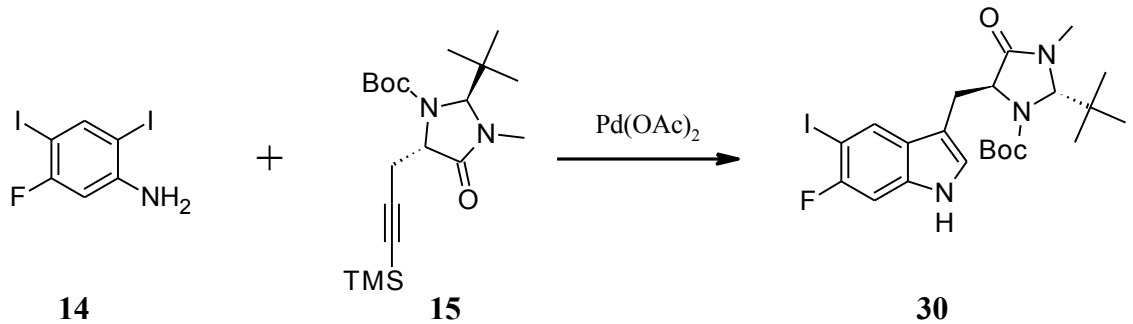


27 **13**

Scheme 47 Attempts for the iodination of the aniline **27**;
i) $NaHCO_3$, I_2, H_2O; ii) $NaIO_4$, KI, NaCl, $AcOH/H_2O$.

Furthermore, a method for the iodination of fluorobenzenes was examined that has been successfully used by Layek et al..[187] Hereby, sodium bicarbonate in combination with elemental iodine in water was used. As well as under the conditions tested before decomposition of the starting material was detected, but not the formation of the iodoaniline **13** which was possibly also due to cleavage of the dithiolan group.

Due to the fact that the previous results for the synthesis of a suitable *ortho*-iodoaniline failed, the diiodoaniline **14** was used for the coupling with the Seebach alkyne **15** in order to prepare a precursor for the radiosynthesis of a L-tryptophan derivative that could possibly be formylated by an iodine-metal exchange and thereby generate an aromatic system that is suitable for a nucleophilic ^{18}F-for-^{19}F isotopic exchange (Scheme 48).



14 **15** **30**

Scheme 48 Pd-catalyzed synthesis of the L-tryptophan derivative **30**.

The Pd-mediated coupling itself was done following a modified procedure to the one previously described by Ma et al..[179] In the original method only monoiodinated fluoroiodoanilines and Schöllkopf functionalized alkynes have been used for this reaction while this reaction should be examined with a diiodinated *ortho*-iodoaniline which has not been investigated before.[179] Since the original setup with $Pd(OAc)_2$, LiCl and Na_2CO_3 in

DMF and heating to 100 °C for 20 h did not give any of the desired product, but a large amount of side products instead, the method was altered regarding reagents, solvent and temperature. Hereby, an increase of temperature to 120 °C did not change the obtained results as well as a change of the solvent from DMF to NMP (*N*-methyl-2-pyrrolidone) and rising the temperature to 140 °C. The base was also changed from Na_2CO_3 to Cs_2CO_3 but again none of the desired product could be achieved. However, the desired compounds could not be obtained regardless the applied conditions. The investigated setups are summarized in Table 7. Since the desired compound **30** could not be isolated the reaction was performed with 2-iodoaniline which has been used by Ma et al. and gave high yields of about 87 % of the corresponding tryptophan analogue. This was done in order to verify that the alkyne **15** which was functionalized with Seebach's auxiliary instead of Schöllkopf's is suitable for this reaction and whether the observed side products are due to the diiodinated iodoaniline **14** or to the alkyne **15**.

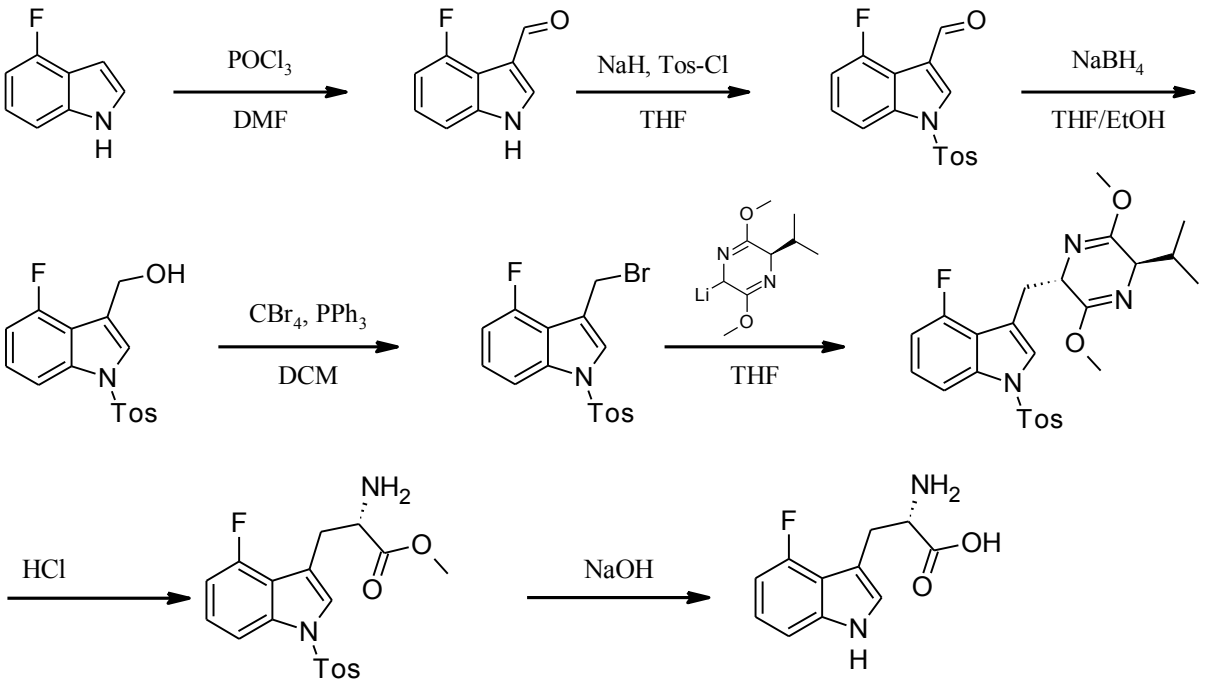
Table 7 Conditions tested for the Pd-mediated formation of **30**.

Solvent	Reagents	Temperature [°C]	Time [h]
DMF	LiCl, Na_2CO_3	100	20
DMF	KI, Na_2CO_3	100	20
DMF	LiCl, Cs_2CO_3	100	20
DMF	LiCl, Na_2CO_3	120	20
NMP	LiCl, Na_2CO_3	100	20
NMP	LiCl, Na_2CO_3	140	20

For this purpose, the reaction was performed under the conditions described by Ma et al.[179] giving 6-fluorotryptophan in similar yields of about 78 %. However, this tryptophan analogue was not suitable as precursor for L-6-[^{18}F]fluorotryptophan since there is no ability of the introduction of a formyl group. Furthermore, it was proven that the preparation of tryptophan analogues by this procedure is possible, but is limited to iodoanilines that do not carry a second iodine. Due to the fact that the preparation of a precursor for the radiosynthesis of L-6-[^{18}F]fluorotryptophan failed via this approach a new synthetic pathway had to be developed.

3.4.2 Precursor for the radiosynthesis via a linear synthetic pathway

In 2012 Konas et al.[160] developed a method for the enantioselective synthesis of L-4-fluorotryptophan via a seven step linear synthesis starting from 4-fluorindole.[160] The original synthetic pathway is depicted in Scheme 49. This route was also used here for the synthesis of the standard L-6-fluorotryptophan, but starting from 6-fluoroindole instead of 4-fluoroindole.

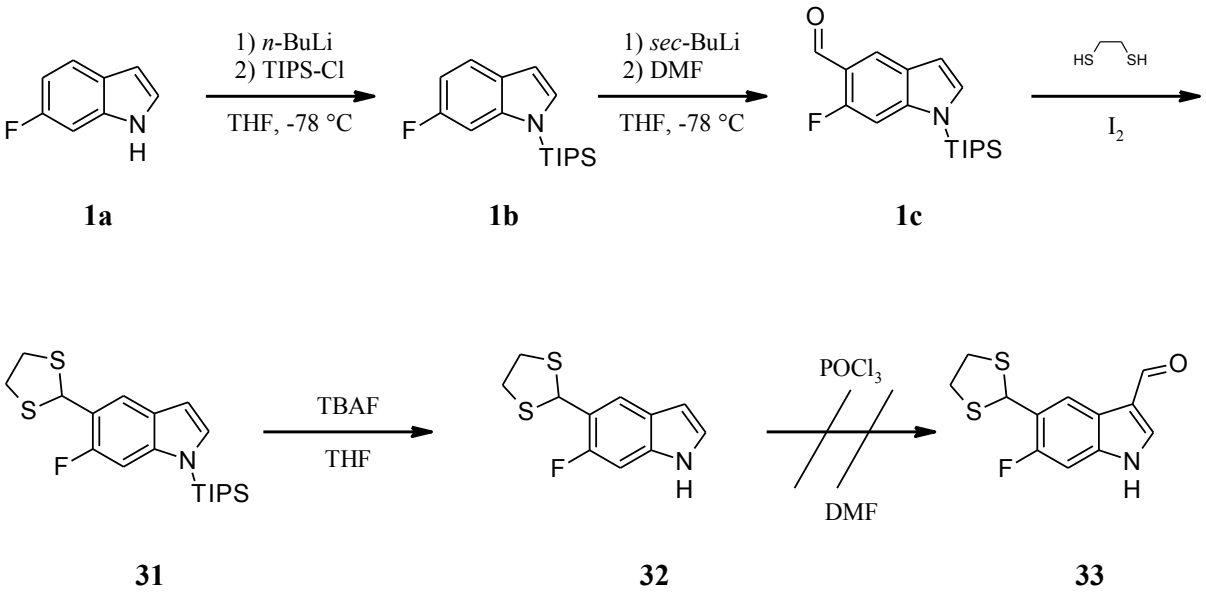


Scheme 49 Original pathway by Konas et al. for the synthesis of L-4-fluorotryptophan.[160]

In order to prepare a precursor that is suitable for a nucleophilic isotopic exchange the introduction of an electron withdrawing group for the activation of the aromatic system was necessary. This should be accomplished by an early introduction of a formyl group *ortho* to the fluorine substituent. The formyl group should be protected subsequently in order to prevent undesired side reactions. In this case 6-fluoroindole was chosen as starting material to realize a precursor for L-6-[^{18}F]fluorotryptophan. An overview of the first steps of the synthetic pathway is given in Scheme 50.

Compound **1c** was prepared following the procedure described in chapter 3.1. The formyl group was then protected with a 1,3-dithiolane group under the conditions used by Firouzabadi et al.[186] yielding the dithiolane **31** in 40 % yield. Here, the dithiolan group was preferred over an oxalane group since oxalanes are labile under acidic conditions and the introduction of a second formyl group later in the pathway in the 3-position was planned through a Vilsmeier-Haack reaction requiring acidic conditions. The deprotection of the

indole nitrogen was done with TBAF giving the free indole **32** in a yield of 92 %. The introduction of the formyl group should be done under standard Vilsmeier-Haack conditions with $POCl_3$ in DMF as published by Smith et al.[84] in order to attach another carbon atom to the indole which could then be further reduced to the alcohol, brominated and alkylated according to the procedure described by Konas et al.[160] The formylation under Vilsmeier-Haack conditions, however, was not successful, and the desired formylindole **33** could not be obtained due to decomposition of the starting material, which was probably due to the dithiolane group, since the reaction proceeded in good yields during the synthesis of the standard L-6-fluorotryptophan.

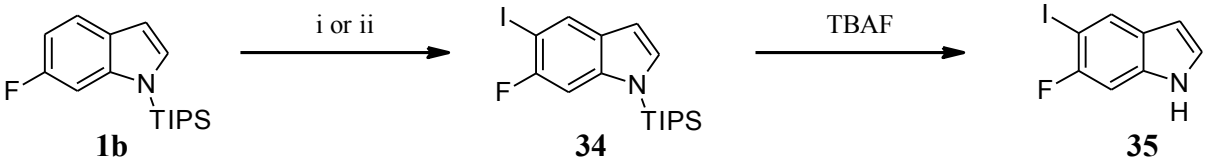


Scheme 50 Attempt for the synthesis of a precursor which is suitable for the radiosynthesis of L-[^{18}F]fluorotryptophan by isotopic exchange.

Since a suitable precursor for the radiosynthesis of [^{18}F]fluorotryptophan was not possible via this approach, the route shown above was altered in a way that the formyl group in the indole **1c** was substituted by an iodine which can be converted into a formyl group by a halogen-metal exchange at a later stage of the whole synthesis and thereby generating a molecule that is more stable towards a wide range of reaction conditions. The introduction of the iodine was first done following a procedure described by Schlosser et al.[69] using *sec*-BuLi in combination with *N*,*N*,*N*',*N*'',*N*''-pentamethyl-diethylenetriamine (PMDTA) for the de-protonation of the indole and elemental iodine as electrophile for the iodination (Scheme 51).

Due to the fact that separation of the compounds **1b** and **34** was not possible, the deprotection with TBAF was always done with crude **34** followed by separation of the desilylated

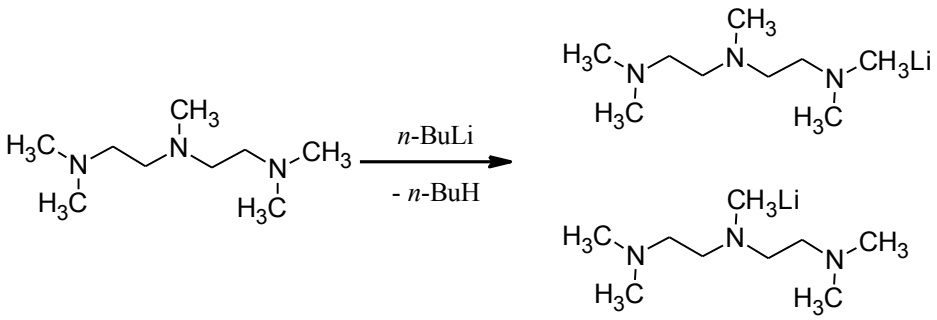
compounds **1a** and **35** by column chromatography. The yields were calculated over both steps, i.e. iodination and deprotection.



1b **34** **35**

Scheme 51 Iodination and deprotection of **1b**;
i) *sec*-BuLi, PMDTA, I_2, THF; ii) *sec*-BuLi, PMDTA, 1,2-diiodoethane, THF.

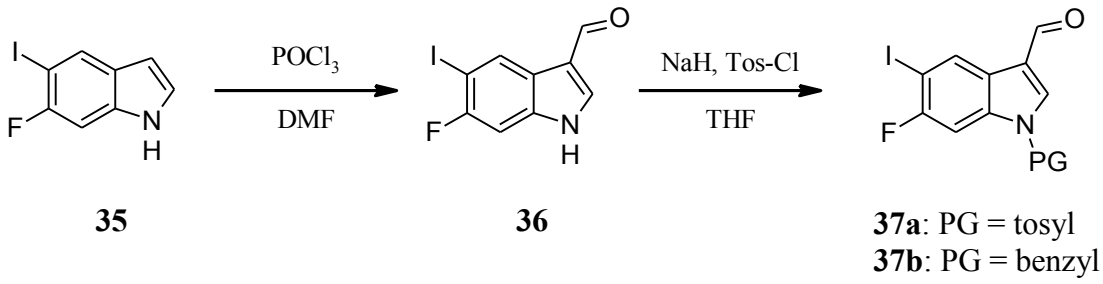
The iodination of the fluoroindole **1b** is a crucial step in the synthetic sequence of the precursor. For this reaction the addition of equimolar amounts of PMDTA was essential. PMDTA is commonly used in organic synthesis for the activation of organolithium compounds, such as *n*-BuLi or *sec*-BuLi. Usually simple alkyllithum compounds occur as oligomers (e.g. hexameric *n*-BuLi) in solution. This inhibits or prevents precoordination to the reactive site of other reagents. Through the addition of bi- or tridentate ligands such as PMDTA those oligomers are broken up and reactive monomeric lithium-species are formed (Scheme 52).[188]



Scheme 52 Lithiation of the central and terminal methyl groups of PMDTA with *n*-BuLi.

When the reaction was performed without the addition of equimolar amounts of PMDTA the desired free iodoindole **35** could not be obtained and the starting material, 6-fluoroindole, was recovered. Furthermore, it was necessary that the reaction was kept at -78 °C for at least six hours after the addition of *sec*-BuLi. Otherwise decreasing yields were obtained while keeping the reaction mixture at -78 °C for more than six hours did not result in higher yields. In the beginning elemental iodine was used as electrophile for this reaction giving the desired iodoindole **35** in about 65 % yield after deprotection and purification. Later, the reaction was

carried out using 1,2-diiodoethane as iodination agent which resulted in similar yields but a much easier handling of the reaction.



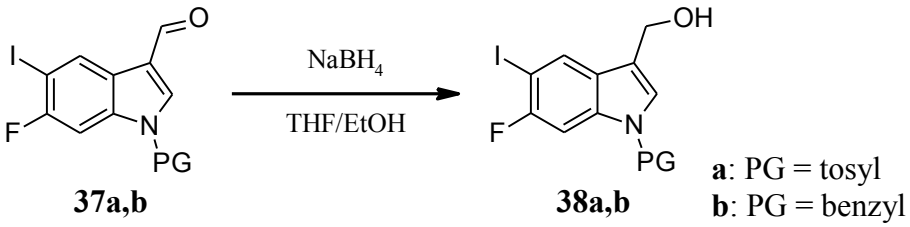
35 **36** **37a**: PG = tosyl
37b: PG = benzyl

Scheme 53 Preparation of 6-fluoro-5-iodo-1*H*-indole-3-carbaldehyde (**37a,b**).

The next step in the synthesis was the formylation of the iodoindole **35** in the 3-position. This was done under standard Vilsmeier–Haack conditions[84] giving the indole-3-carbaldehyde **36** in 64 % yield.

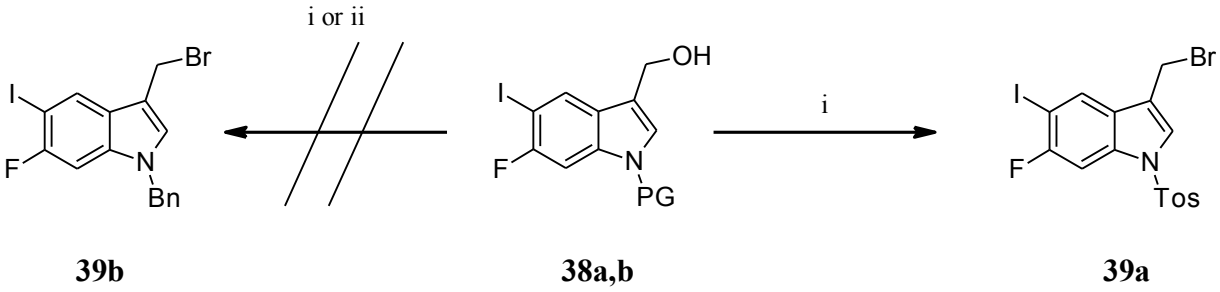
For the protection of the indolecarbaldehyde **36** two different protecting groups were studied. On one hand a tosyl group was selected which was also chosen by Konas et al. and should be suitable for the following reactions. On the other hand the previous studies on the radiofluorination of the indolecarbaldehydes in chapter 3.2 have shown that the best RCY was obtained when the nitrogen of the indole was protected with a benzyl group. In order to avoid additional steps for the change of protecting groups later in the synthetic pathway, the indolecarbaldehyde **36** was alternatively protected with both protecting groups, yielding the tosyl- and benzyl-protected indolecarbaldehydes **37a** and **37b** in 81 % and 60 % yield, respectively. It was also promising that the benzyl group could be removed after the radiosynthesis since the 3-position was occupied in the precursor which should allow deprotection with $AlCl_3$ in benzene or another aromatic solvent.

The following reduction of the indolecarbaldehydes **37a** and **37b** was done with $NaBH_4$ in a mixture of THF and EtOH (Scheme 54) giving **38a** in quantitative yields while **38b** could only be obtained in 65 % yield.



Scheme 54 Reduction of the indolecarbaldehydes **37a** and **37b** to the corresponding alcohols **38a** and **38b**.

In order to enable an alkylation with Seebach's auxiliary the alcohol should be converted into a bromide. This was done via an Appel reaction using CBr_4 and PPh_3 in DCM.[189] Using this procedure the tosyl protected bromine **39a** could be synthesized in 56 % yield when the reaction temperature was kept at 0 °C and not allowed to get higher than 25 °C until the bromide was separated from triphenylphosphine oxide which is formed during the reaction. Otherwise isolation of the desired product **39a** was impossible due to instability

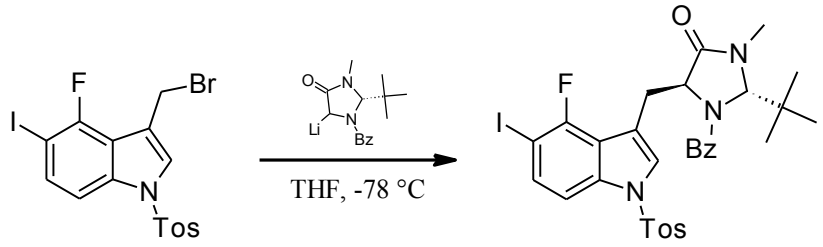


Scheme 55 Bromination of the indolylalcohols **38a** and **38b;**
i) PPh_3, CBr_4, DCM, 0 °C; ii) PBr_3, DCM.

Applying the conditions described above to the benzyl protected alcohol **38b** resulted in decomposition of the starting material and formation of a wide range of side products even when the reaction temperature was reduced to -20 °C. Furthermore, the reaction was performed in DCM using PBr_3 as bromination agent[190] but those conditions also resulted in decomposition. Later it was found that compound **38b** shows high instability when dissolved in chlorinated solvents such as DCM, chloroform or carbon tetrachloride, which makes bromination of this molecule impossible, since aliphatic bromine for hydroxyl nucleophilic substitution reactions are usually realized in chlorinated solvents. Hence, compound **39b** could not be synthesized successfully.

Therefore, the synthetic pathway was continued with the tosyl protected bromine **39a**. Hereby, it was important that the alkylation with Seebach's auxiliary needs to be done directly after purification of the bromide **39a** because otherwise it was subject to slow decomposition, especially when exposed to light.

The introduction of the chiral center by alkylation with Seebach's auxiliary was carried out under standard conditions at –78 °C in THF using LDA as base and proceeded with 73 % yield giving the iodofluoroindole **40** (Scheme 56).

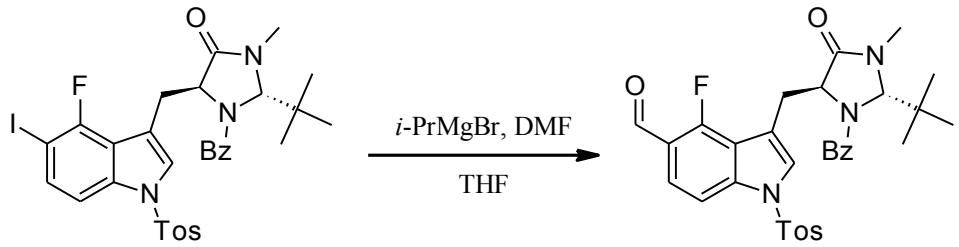


39a **40**

Scheme 56 Synthesis of (2S)-2-tert-butyl-5-[(4-fluoro-5-iodo-1-methyl-1*H*-indol-3-yl)methyl]-1,3-dimethylimidazolidin-4-one (**40**).

Further on, the aldehyde function was introduced which is necessary for the activation of the carbocycle for the planned isotopic exchange. This aim was accomplished by a procedure previously described by Boymond et al.[191] and Armstrong et al.[192]. The method based on a Grignard reaction with *i*-PrMgBr which mediates the iodine-magnesium exchange followed by the addition of DMF (Scheme 57). *i*-PrMgBr is commonly used in Grignard-like reactions for iodine-magnesium exchanges. Reactions where *i*-PrMgBr is applied instead of elemental magnesium can generally be carried out under milder conditions which results in the formation of less side products and often higher yields.

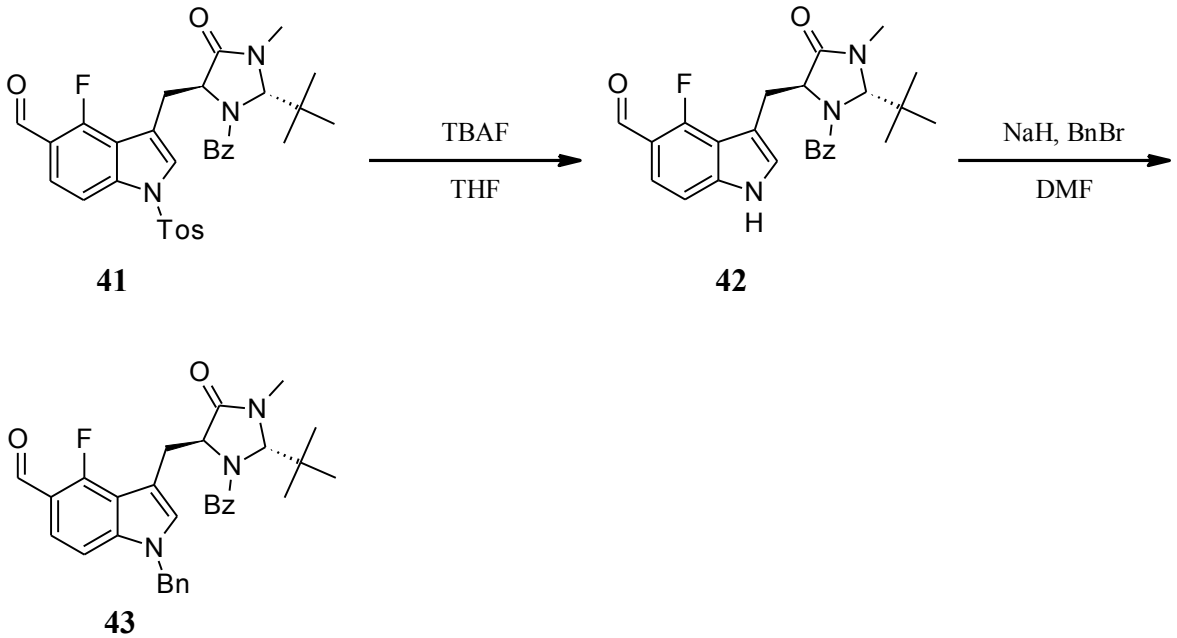
Performing the reaction under the conditions described by Boymond et al.[191] gave the corresponding aldehyde **41** in 78 % yield. During the formylation procedure it was essential to use at least four equivalents of DMF, because otherwise only yields of less than 50 % were achieved.



40 **41**

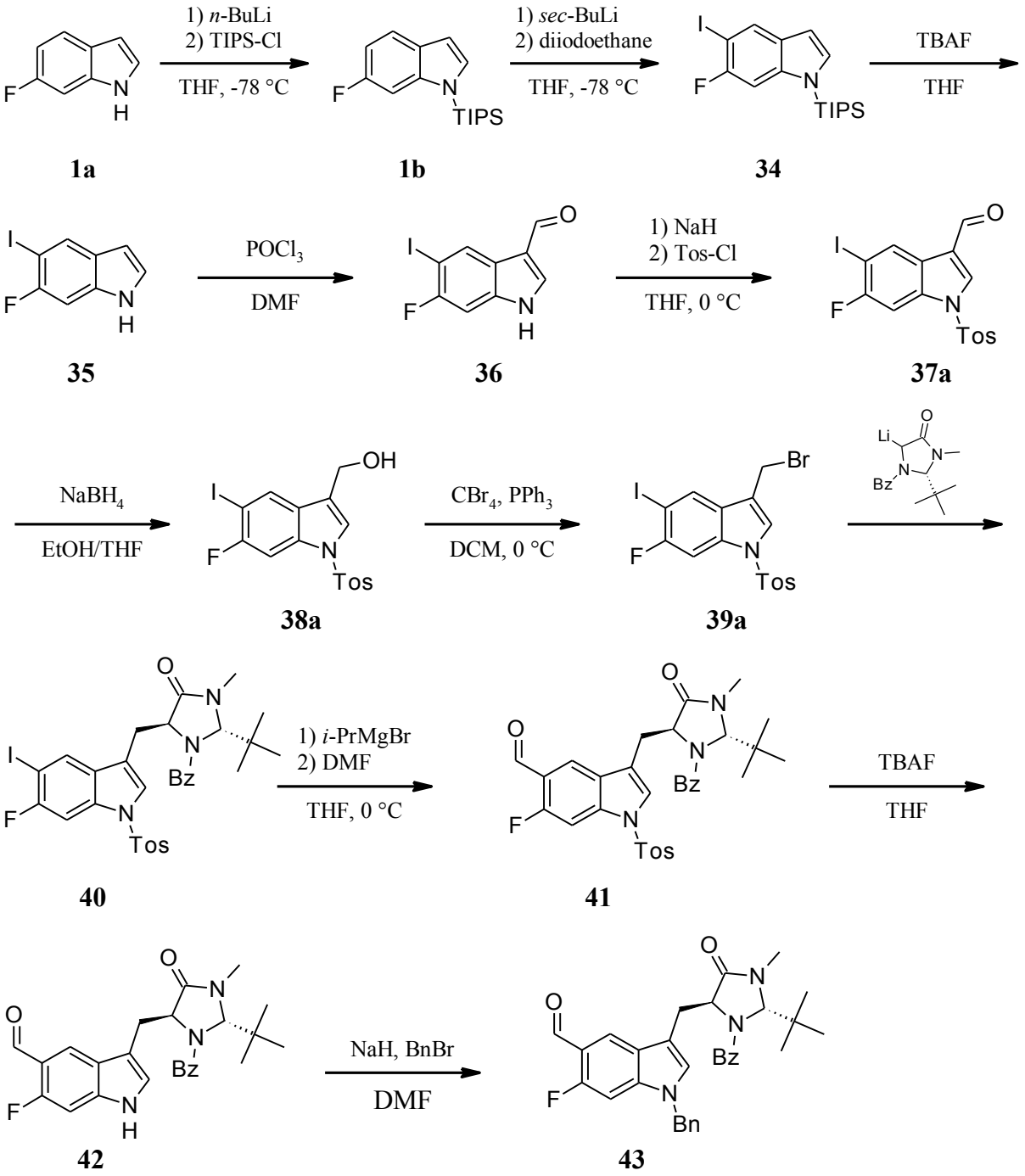
Scheme 57 Formylation of **40** with *i*-PrMgBr and DMF.

The final steps in the synthetic pathway of the precursor were the removal of the tosyl group and the protection of the indole nitrogen with a benzyl group which gave the best RCY when studied on the indole-1*H*-carbaldehydes (see chapter 3.2). The detosylation was done according to a procedure described by Yasuhara et al.[170] using TBAF in THF for this purpose. The reaction was performed at room temperature giving the desired free indolecarbaldehyde **42** in 91 % yield (see Scheme 58). The subsequent benzylation was carried out following the procedure that was also used for the benzylation of the indole-1*H*-carbaldehydes in chapter 3.1 giving the precursor for L-6-[^{18}F]fluorotryptophan (**43**) in 78 % yield. Finally, a potential precursor for the radiosynthesis of L-6-[^{18}F]fluorotryptophan could be synthesized by an eleven step linear synthesis in an overall yield of 7 %. An overview of the synthetic sequence is shown in Scheme 59.



41 **42** **43**

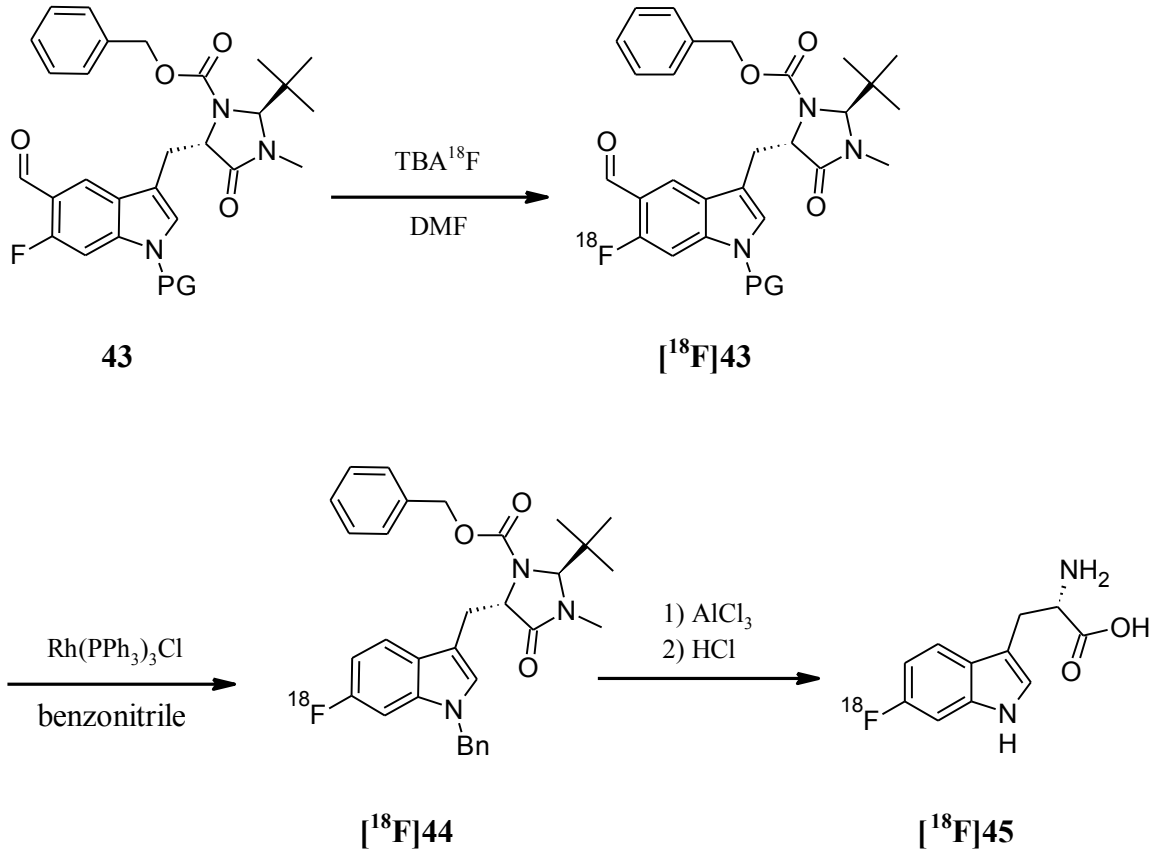
Scheme 58 Final reaction steps for precursor of L-6-[^{18}F]fluorotryptophan **43.**



Scheme 59 Overview of the elaborated (11 step) synthetic pathway of a precursor for the radiosynthesis of L-6-[^{18}F]fluorotryptophan (**43**) by isotopic exchange.

3.5 Radiosynthesis of L-6-[^{18}F]fluorotryptophan

The radiosynthesis of L-6-[^{18}F]fluorotryptophan (**[^{18}F]45**) was planned to proceed in three steps, isotopic exchange, reductive decarbonylation and removal of protecting groups according to a modified procedure previously used by Castillo et al.[173] for the radiosynthesis of aromatic amino acids (Scheme 60).



43 **[^{18}F]43**

[^{18}F]44 **[^{18}F]45**

Scheme 60 Synthetic concept for the radiosynthesis of L-6-[^{18}F]fluorotryptophan **[^{18}F]45**.

The first step in this route was the introduction of the [^{18}F]fluoride by an isotopic ^{18}F-for-^{19}F isotopic exchange. This was done under the optimum conditions elaborated for the isotopic exchange on 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**) which has a similar substitution pattern. Therefore, the reaction was first examined at 150 °C in DMF with $TBAHCO_3$ as anion activator.

Since the tosyl-protected precursor **41** was available through the same pathway as the benzyl protected precursor **43** its radiofluorination was examined which would shorten the synthesis of the precursor by two steps. Unfortunately, radiolabeling of tosyl-protected **41** could not be obtained under any conditions studied. The applied temperatures that were investigated for

this purpose reached from 120 – 150 °C. No radiofluorinated product could be detected at 120 °C while at 150 °C an unpolar product was found which could be identified as tosyl-[^{18}F]fluoride. This was not surprising since detosylation has been observed before as fluoride is also used for the detosylation of indoles, but usually a large excess of fluoride is used for this purpose.

In first radiolabeling experiments using the benzyl-protected precursor **43** at 150 °C a RCY of only 9±4 % could be found. Furthermore, racemization was observed during the radiofluorination at this temperature giving the L- and D- diastereomer of **[^{18}F]43** in a ratio of about 70:30. Also, several radioactive side products could be detected. When temperatures lower than 150 °C were applied the desired radiolabeled product **[^{18}F]43** could not be obtained at all.

A reductive decarbonylation step was also examined under the conditions that gave the best RCY for 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**) (see chapter 3.2.2) with 3 equivalents of Wilkinson's catalyst and microwave assisted heating (100 W, 2min) in benzonitrile. Thereby, however, the decarbonylated **[^{18}F]44** could be obtained in a RCY of 11±2 % along with several side products, that were not further characterized while this reaction gave yields of > 95 % when it was done on the indolecarbaldehydes **[^{18}F]1e**, **[^{18}F]2e**, **[^{18}F]3e** and **[^{18}F]4e** under the conditions applied here.

The final step in the radiosynthesis was the removal of the benzyl group which was first tested under acidic conditions using conc. HCl and heating to 150 °C for 30 min. This resulted in decomposition of **[^{18}F]44** while free 6-[^{18}F]fluorotryptophan **[^{18}F]45** could not be observed. Furthermore, the debenzylation reaction was performed according to the procedure of Murakami et al.[176] with $AlCl_3$ in benzene followed by removal of the solvent and an acidic hydrolysis of the Seebach group with conc. HCl at 150 °C for 30 min. But under those conditions neither the desired product L-6-[^{18}F]fluorotryptophan **[^{18}F]45** nor the starting material **[^{18}F]44** could be detected.

In summary, L-6-[^{18}F]fluorotryptophan (**[^{18}F]45**) could not be obtained from the precursor **43** via the synthetic pathway shown in Scheme 60. This is due to the fact that the benzyl protecting group and thereby the hydrolysis of the protecting groups did not succeed. Though, the isotopic exchange on **43** and the reductive decarbonylation on **[^{18}F]43** were possible even if only a low RCY of about 9 % and 11 %, respectively, was obtained. This is probably due to the low reactivity for precursor **43** towards the isotopic exchange that was also observed with 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**) and different to the other indolecarb-

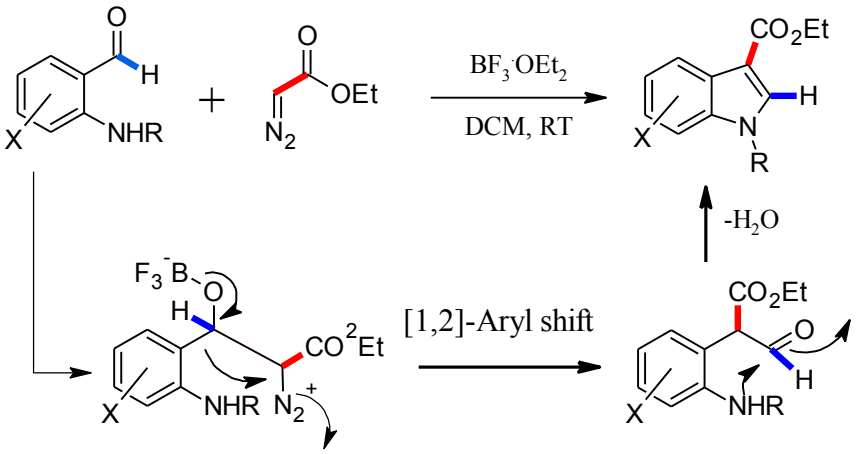
aldehydes (**2e**, **3e** and **4e**). These results confirm those ones obtained in chapter 3.2. For this reason a new precursor needed to be prepared carrying the fluorine in the more activated 4-position and the formyl group in the 5-position, if possible, since this substitution pattern gave by far the best RCY on the isotopic exchange and showed the best properties regarding chemical stability. Furthermore, labeling of fluoro-1*H*-indolecarbaldehydes with this substitution pattern gave already the maximum RCY at temperatures of about 90 C. Hereby, it might be possible to avoid racemization or at least to minimize it.

3.6 Synthesis of precursor for the radiosynthesis of L-4-[^{18}F]fluorotryptophan

In addition to the precursor for 6-fluorotryptophan a synthetic pathway should be developed for the synthesis of a precursor that is suitable for the radiosynthesis of 4-[^{18}F]fluoro-tryptophan since this substitution pattern gave high RCY when it was tested on the fluoro-1*H*-indolecarbaldehdes, as described in chapter 3.2.

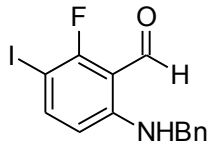
3.6.1 Precursor for the radiosynthesis via build-up synthesis

In 2012 Levesque et al. published a new method for the preparation of substituted indoles by an [1,2]-aryl shift condensation.[193] The starting materials for this are an alkylated amino-benzaldehyde and ethyl diazo acetate. The reaction mechanism is shown in detail in Scheme 61.



Scheme 61 Mechanism of the preparation of indoles via an [1,2]-aryl shift condensation as proposed by Levesque et al. [193]

Ethyl diazoacetate is commercially available but the functionalized aminobenzaldehyde needs to be prepared prior to the cyclization reaction. The aminobenzaldehyde of choice is shown in Figure 10. The positions of the fluorine and iodine were chosen in order to generate a 4-fluoroindole where a formyl group can be easily introduced in the 5-position, since this substitution pattern gave the best RCY in the experiments described in chapter 3.2. A benzyl group was chosen as protecting group for the amine since benzyl protected aminobenzaldehydes gave the best yields in the indole formation described be Levesque et al.[193] Furthermore, the best RCY on the isotopic exchange could also be achieved with 1-benzyl-4-fluoro-1*H*-indole-5-carbaldehyde (**2e**).

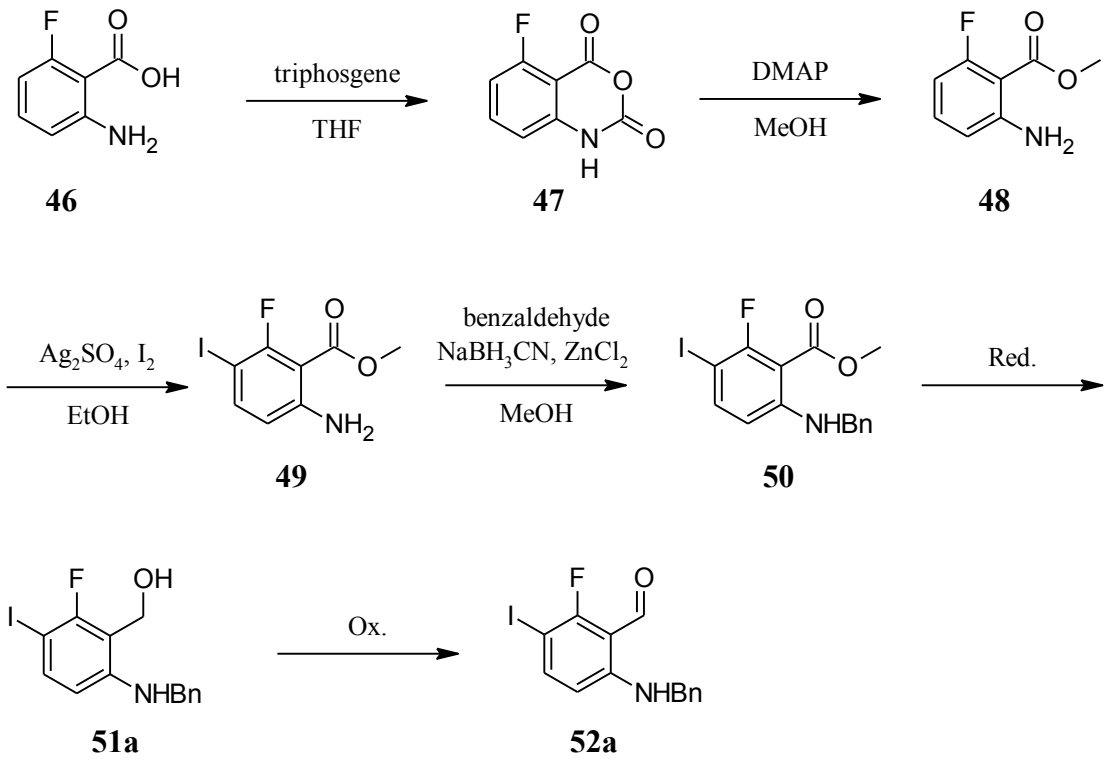


52a

Figure 10 6-(Benzylamino)-2-fluoro-3-iodobenzaldehyde as building block for the indole formation via the method described by Levesque et al.

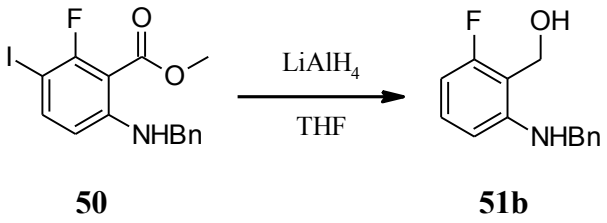
A route for the synthesis of the amino benzaldehyde (**52**) was developed starting from 2-amino-6-fluorobenzoic acid (**46**) which is shown in Scheme 62.

The first step of this procedure was the cyclisation of the aminobenzoic acid **46** which was previously done by Tedesco et al.[194] with phosgene in THF. Due to the fact that phosgene is highly toxic and difficult to handle, since it is a gas, triphosgene was used instead giving also quantitative yields of **47**. The methyl ester **48** was prepared by refluxing **47** in ethanol with a catalytic amount of DMAP in 97 % yield. The iodination of **48** was realized following a method described by DeVita et al.[195] using elemental iodine and Ag_2SO_4 in ethanol. Thereby methyl 6-amino-2-fluoro-3-iodobenzoate (**49**) could be obtained in 80 % yield. The benzylation of the amine was carried out by a reductive amination with benzaldehyde and $NaBH_3CN$ following a procedure described by Levesque et al.[193] who performed this reaction in methanol at room temperature. This approach, however, did not give the desired compound **50**. Refluxing was necessary to convert the free amine into the corresponding benzylamine in about 78 % yield. The following reduction of the methyl ester **50** was first studied under standard conditions using $LiAlH_4$ in THF followed by an acidic workup.



Scheme 62 Synthetic pathway for the preparation of the aminobenzaldehyde **52**.

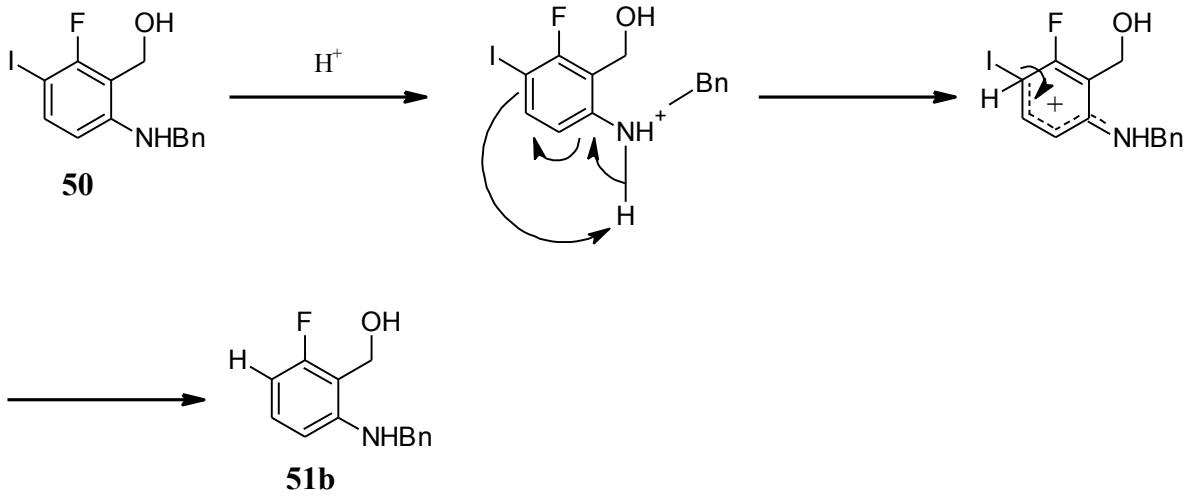
Unfortunately, the application of these conditions led to the formation of the deiodinated alcohol **51b** (Scheme 63). This might be due to a mechanism proposed by Twum et al.[196] who also observed aromatic deiodination when *ortho*- or *para*-iodoanilines were handled under acidic conditions. The proposed mechanism is shown in Scheme 64 at the example of the deiodination of **50**. An alternative explanation for the deiodination might be an electrophilic retroiodination which would result the same compound **51b**.



Scheme 63 Reduction of **50** with $LiAlH_4$ followed by an acidic workup yielding the deiodinated benzyl alcohol **51b**.

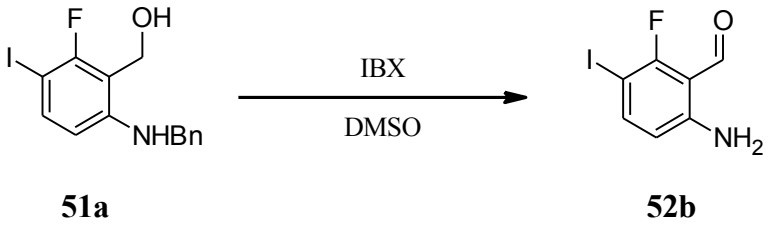
In order to avoid acidic conditions during work-up the reaction was quenched rather with water than with 1M HCl. The resulting slurry was filtered and intensively washed with DCM

to get the desired benzyl alcohol **51a** in 57 % yield. The yield could be increased to 85 % after purification when DIBAL (diisobutylaluminiumhydride) was used as reducing agent. This is probably due to the fact that DIBAL forms a colorless solid when water is added which can be filtered off more easily.



Scheme 64 Mechanism proposed by Twurm et al. for aromatic deiodination under acidic conditions at the example of **51b**.[196]

The following oxidation of the benzyl alcohol **51a** was first performed with IBX (2-iodoxybenzoic acid), a hypervalent iodine oxidant that is commonly used for the oxidation of alcohols to aldehydes.[197],[198] The reaction itself was carried out under the conditions used by Levesque et al.[193] Unfortunately, when these conditions were applied, not only the alcohol was oxidized to the corresponding benzaldehyde, but also the benzyl group on the amine was removed giving the aminobenzaldehyde **52b** in 78 % yield (Scheme 65). When Dess-Martin-periodane (DMP), a derivative of IBX, was used, decomposition of the starting material occurred, which was probably due to the higher reactivity of DMP.

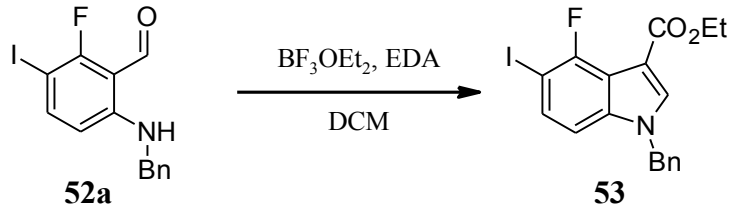


51a **52b**

Scheme 65 Oxidation of the benzyl alcohol **51a** with IBX resulting in the debenzylated aminobenzaldehyde **52b.**

Finally, activated manganese dioxide (MnO_2) was applied as oxidant which is also a common reagent for the oxidation of benzyl alcohols to benzaldydes under extremely mild conditions. Usually an excess of MnO_2 is used and DCM is the solvent of choice. With this procedure the desired benzaldehyde **52a** was obtained in about 67 % yield.

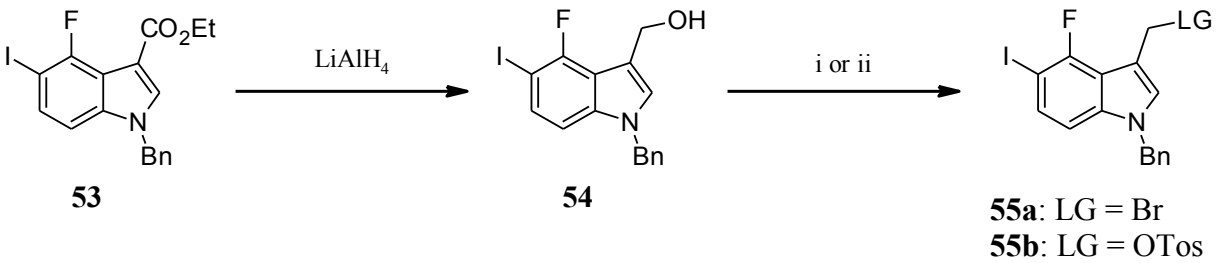
Next, the prepared benzaldehyde **52a** was reacted with ethyl diazoacetate under the conditions described by Levesque et al. giving the desired fluoro-iodo-indole **53** in 83 % yield (Scheme 66).



Scheme 66 Preparation of the fluoroiodoindole **53.**

Since the preparation of **53** was successful and could be accomplished with high yields, the ester in the 3-position of the indole should be reduced to the corresponding alcohol followed by the introduction of a leaving group and the alkylation with Seebach's auxiliary. The reduction was first tried with $LiAlH_4$ in THF. The alcohol **54** could be detected by TLC but during purification decomposition already occurred. Due to the observed instability of the alcohol **54** it was directly used for the next reaction step without further purification.

When the conversion of the alcohol **54** into the corresponding bromide (**55a**) or tosylate (**55b**) was examined, decomposition of the starting material occurred immediately and none of the compounds could be isolated.

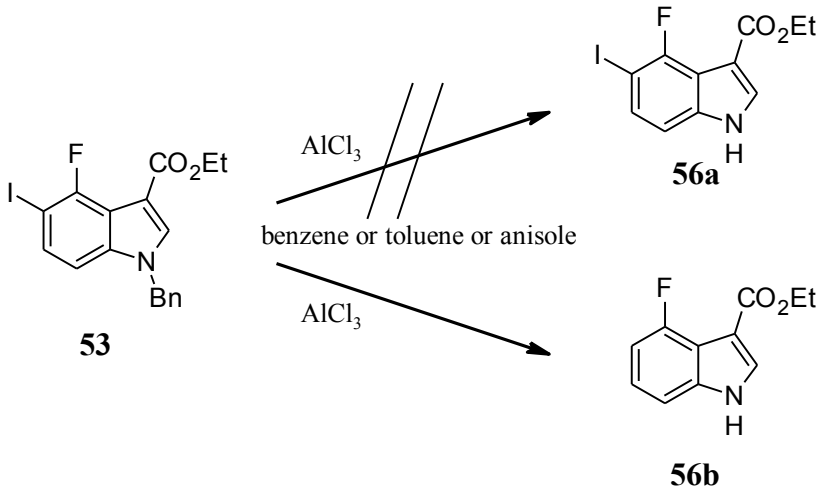


Scheme 67 Attempts for the preparation of the compounds **55a,b**, i) CBr_4, PPh_3, DCM; ii) NEt_3, Tos-Cl, MeCN**.**

The bromination of the instable alcohol **54** was done by an Appel reaction at 0 °C resulting in decomposition of the starting material as soon as it was dissolved in DCM. This was similar to the benzyl protected indole **38b** described in chapter 3.4.2. Therefore, a tosylation was attempted in acetonitrile with triethylamine as base, since decomposition of benzyl-protected indoles was not observed under those conditions. Using these conditions, neither the starting material nor the tosylate **55b** could be obtained due to immediate decomposition of the labile alcohol **54**.

Since the introduction of a suitable leaving group for the alkylation with Seebach's auxiliary was impossible, the benzyl group should be removed and substituted by a protecting group, in another attempt, resulting in a compound that is stable under the conditions required for the following reactions. In this case a tosyl-group should be introduced which indeed worked well for bromination of the alcohol **38a**.

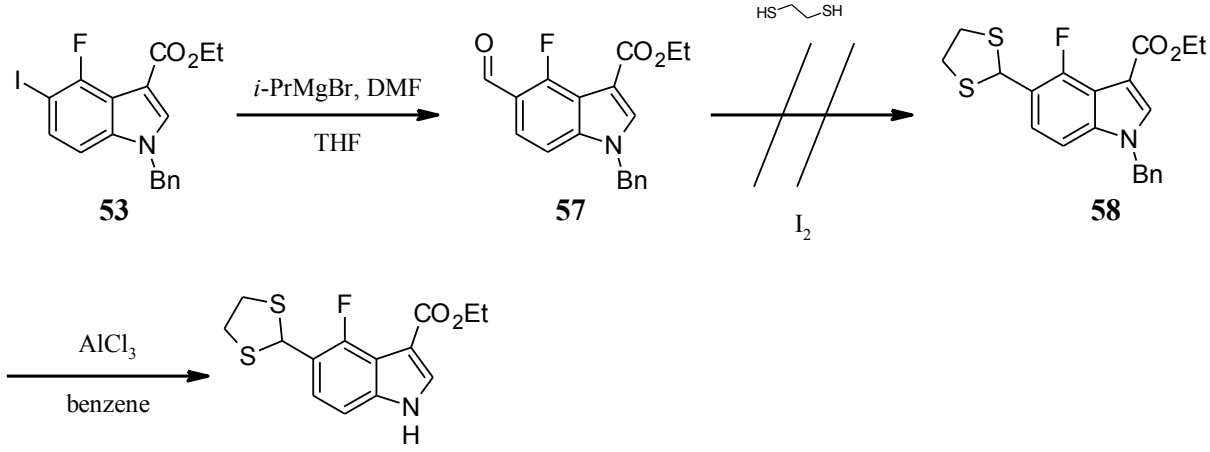
The removal of the benzyl group was performed under the conditions previously described by Murakami et al. using 5 equivalents of $AlCl_3$ in benzene.[176] When these conditions were applied, the debenzylated indole **56a** could not be obtained. Instead, the formation of the debenzylated and deiodinated indole **56b** was observed which could be isolated in 78 % yield (Scheme 68). In order to avoid the deiodination during the debenzylation step the impact of the solvent on the deiodination was tested. Therefore, toluene and anisole were also tested as solvents for this reaction since they have been used previously for those kinds of reactions.[177],[158] Inopportunely using these solvents gave the same results that have been observed before.



Scheme 68 Debenzylation of **53** using $AlCl_3$ in various solvents.

Moreover, it was studied whether the use of one equivalent of $AlCl_3$ is effective for the debenzylation of **53**. This was done in order to find out whether the benzyl-group is more reactive to $AlCl_3$ than the iodine. If this was the case, debenzylation would be possible without deiodination, which would potentially result in longer reaction times while yielding the desired **56a**. Since no conversion could be observed with one equivalent, the amount of $AlCl_3$ was increased slowly in order to find a ratio of starting material and reagent where the desired debenzylation proceeds, but the undesired deiodination does not. This was not possible, since debenzylation was not observed without deiodination. Therefore, the removal of the benzyl group was examined under various conditions. First, the reaction was carried out with Pd/C and NH_4HCO_2 in EtOH which did not result in any conversion of the starting material. The same results were obtained when Pd/C and the benzyl protected indole **53** were dissolved in MeOH and hydrogen gas was passed through the solution. However, it was impossible to obtain the debenzylated iodoindole **56a** and it was studied whether the formylation of the benzylinolde **53** is possible followed by protection of the aldehyde and debenzylation of the indole nitrogen (Scheme 69).

The formylation was done by an iodine-magnesium exchange under the conditions previously described by Boymond et al.[191] starting from the iodoindole **53** using *i*-PrMgBr and DMF at 0 °C. Due to the fact that these conditions led again to fast decomposition of the starting material, the reaction was carried out with the same reagents at -78 °C. Thereby, the desired indolecarbaldehyde could be obtained in 25 % yield. The following protection was tried under standard conditions[186] with 1,2-ethandithiol in DCM and catalytic amounts of elemental iodine. Unfortunately, decomposition of the starting material started immediately after it was dissolved in DCM as it has been observed before for compound **53**.



Scheme 69 Planned synthetic pathway for the formylation and debenzylation of **53**.

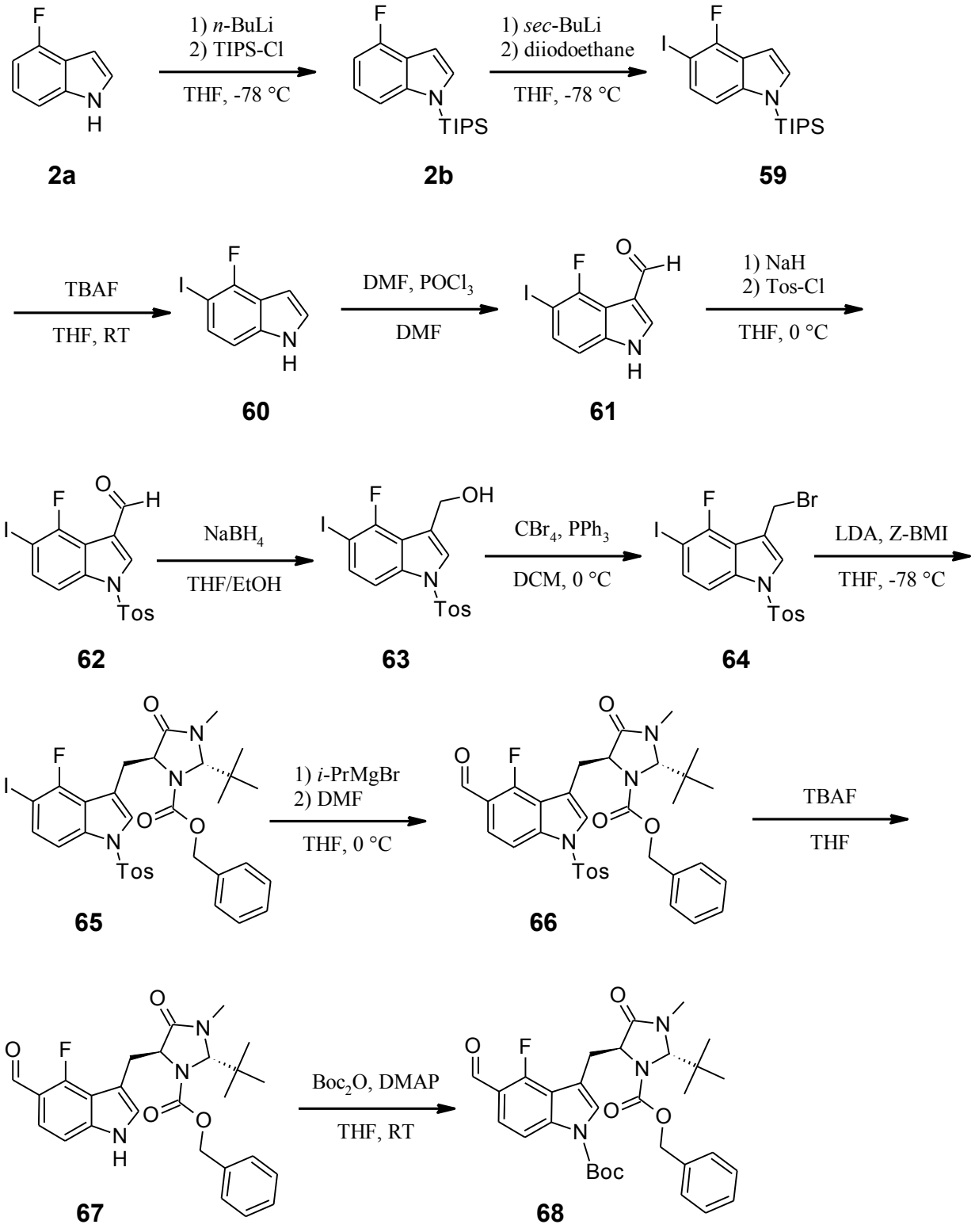
These observations are similar to those that were made when the benzyl protected indole **38b** was dissolved in DCM and might be a further evidence for the instability of some benzyl protected indoles in DCM. Due to the complications that come along with this route, especially the debenzylation and the reduction, it was not as promising as previously assumed and therefore not further investigated.

3.6.2 Precursor for the radiosynthesis via a linear synthetic pathway

The experiments in context of a linear synthesis of a precursor for L-6-[^{18}F]fluorotryptophan (**43**) revealed to be a good strategy. Since 4-fluoro-1*H*-indole (**2a**) became commercially available at reasonable prices, the synthetic concept developed in chapter 3.3.2 could be adapted to the synthesis of a suitable precursor for L-4-[^{18}F]fluorotryptophan starting from 4-fluoro-1*H*-indole here.

The whole synthetic route of the precursor of L-4-[^{18}F]fluorotryptophan is shown in Scheme 70 (see also Scheme 59, chapter 3.4.2) . The protection of 4-fluoroindole (**2a**) was carried out as discussed in chapter 3.1 giving the desired TIPS-indole **2b** in 94 % yield. The iodination was done with *sec*-BuLi, PMDTA and diiodoethane at -78 °C followed by removal of the TIPS group. This was performed without characterization of the TIPS protected iodoindole **59** since the separation of the iodinated and not iodinated TIPS-protected indoles **59** and **2b** was also impossible for those compounds. The yield for both steps was about 65 %. The Vilsmeier-Haack formylation was realized under standard conditions[84] as described in chapter 3.4.2. In contrast to the 6-fluoroindole-3-carbaldehyde **36** which gave about 64 % yield, the 4-fluoro derivative **61** could be obtained in yields as high as 89 % which has to be due to the substitution pattern of the fluorine on the indole. The following tosylation was accomplished in THF with TosCl and NaH giving the tosylated indolecarbaldehyde **62** in 74 % yield. The reduction was carried out with $NaBH_4$ in THF/EtOH yielding the alcohol **63** quantitatively. Bromination of **63** via an Appel reaction gave the bromide **64** in yields of about 63 %. The bromide **64** was stable at room temperature and not as sensitive to light as the 6-fluoroderivative **39** which had to be used immediately after preparation in order to avoid decomposition. With the bromide **64**, storage at 8 °C was possible for at least 2 weeks which simplified the handling with this compound. The alkylation with Seebach's auxiliary was performed under standard conditions with LDA at -78 °C giving compound **65** in 73 % yield. The following introduction of the formyl group in 5-position was done by an iodine-magnesium exchange with *i*-PrMgBr followed by the addition of DMF, thus, yielding the

resulting formylindole **66** in about 73 % yield. Hereby, the use of four equivalents of DMF was also essential. When less DMF was used the obtained yields were below 50 %.



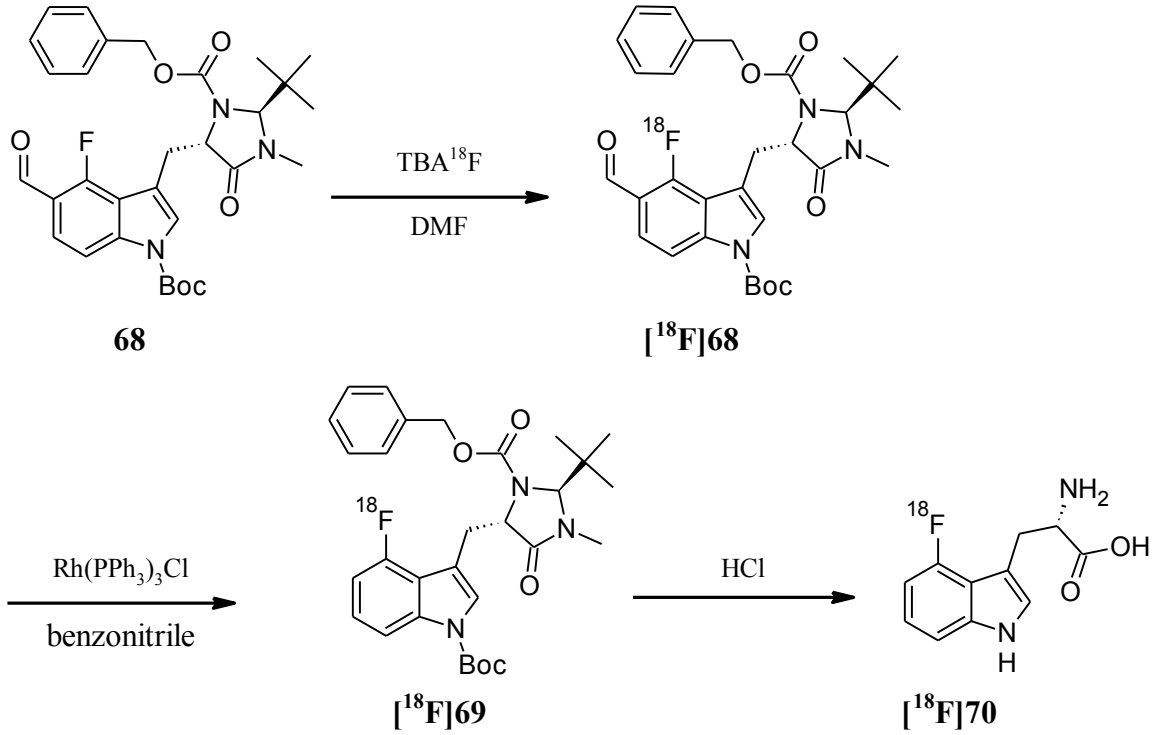
Scheme 70 Synthetic pathway for precursor **68** for the radiosynthesis of L-4-[^{18}F]fluorotryptophan.

The removal of the tosyl group was performed with TBAF in THF at room temperature yielding the unprotected indole **67** in about 42 % besides a variety of side products. This behavior, however, was not observed, when the 6-fluoro derivative **41** was subject to detosylation where yields above 90 % were obtained, and the formation of side products was not observed. Finally, the Boc-group was chosen for the protection of the precursor over the benzyl-group which was used for the protection of the precursor of L-6-fluorotryptophan (**43**) for several reasons. The 1-Boc-4-fluoro-1*H*-indole-5-carbaldehyde (**2g**) which has a similar substitution pattern gave RCY of about 28 % and the removal of the Boc-group can be accomplished under much milder conditions than the removal of a benzyl-group. This might lead to lower RCY in the isotopic exchange, but has some advantages during the planned radiosynthesis. It reduces the radiosynthesis by one step, avoids the removal of the very stable benzyl group as well as the use of $AlCl_3$ and avoids benzene as solvent which is known to be toxic. The introduction of the Boc-group was done with Boc_2O and catalytic amounts of DMAP in THF giving the desired precursor **68** in about 86 % yield.

In summary, a precursor for the radiosynthesis of L-4-[^{18}F]fluorotryptophan was successfully synthesized in an eleven step linear synthesis in an overall yield of about 8 % following the synthetic pathway that has been developed before in this work for the preparation of a similar precursor for L-6-[^{18}F]fluorotryptophan.

3.7 Radiosynthesis of L-4-[^{18}F]fluorotryptophan

The radiosynthesis of L-4-[^{18}F]fluorotryptophan was carried out in three steps following again the concept that was previously developed by Castillo et al.[173] and also used for the attempts of the radiosynthesis of L-6-[^{18}F]fluorotryptophan. The three steps consist of a nucleophilic isotopic ^{18}F-for-^{19}F exchange, a reductive decarbonylation with Wilkinson's catalyst and the hydrolysis of the protecting groups. The whole synthesis sequence is illustrated in Scheme 71.



Scheme 71 Synthetic concept for the radiosynthesis of L-4-[^{18}F]fluorotryptophan (**[^{18}F]70**).

3.7.1 Isotopic exchange

The first step in the radiosynthesis is the isotopic exchange. Since complex molecules containing the indole moiety have not been labeled with nucleophilic fluorine-18 directly in the carbocycle, the reaction was first tried under the conditions that gave the highest RCY for 1-Boc-4-fluoro-1*H*-indole-5-carbaldehyde (**2g**). The best RCY for this compound was obtained at 90 °C where 28 % RCY could be obtained. Therefore, the precursor **68** was reacted for 15 min at this temperature using $TBAHCO_3$ as anion activator and DMF as solvent.[173],[168]

The RCY obtained using the conditions described above was about 17 %. Furthermore, it was examined whether increasing temperatures result in a higher RCY. However, at temperatures higher than 90 °C decomposition occurred. When temperatures higher than 110 °C were used complete decomposition of the somehow temperature sensitive starting material occurred. The isotopic exchange was therefore also investigated at temperatures between 85 °C and 60 °C. The best RCY that could be obtained was about 51 %, and surprisingly the optimum temperature was 80 °C. This was unexpected since it can be found in the literature that isotopic exchange reactions on fluorobenzaldehydes gave the best RCY at temperatures above 100 °C.[168],[169] With temperatures lower than 80 °C the RCY decreased significantly. The temperature region where a good RCY of above 40 % can be obtained is very narrow and is limited from 75 °C to 85 °C. The temperature dependence on the RCY is graphically shown in Figure 11. The isotopic exchange was also tested with DMSO and MeCN as solvents but the RCY obtained thereby were always below 8 % combined with the formation of several side products which was similar to the radiofluorination of the 1-benzyl-fluoro-1*H*-indolecarbaldehydes (**1e-4e**). For this reason, it is suggested to prefer DMF over DMSO and MeCN as solvent for the isotopic fluorine-exchange on indoles.

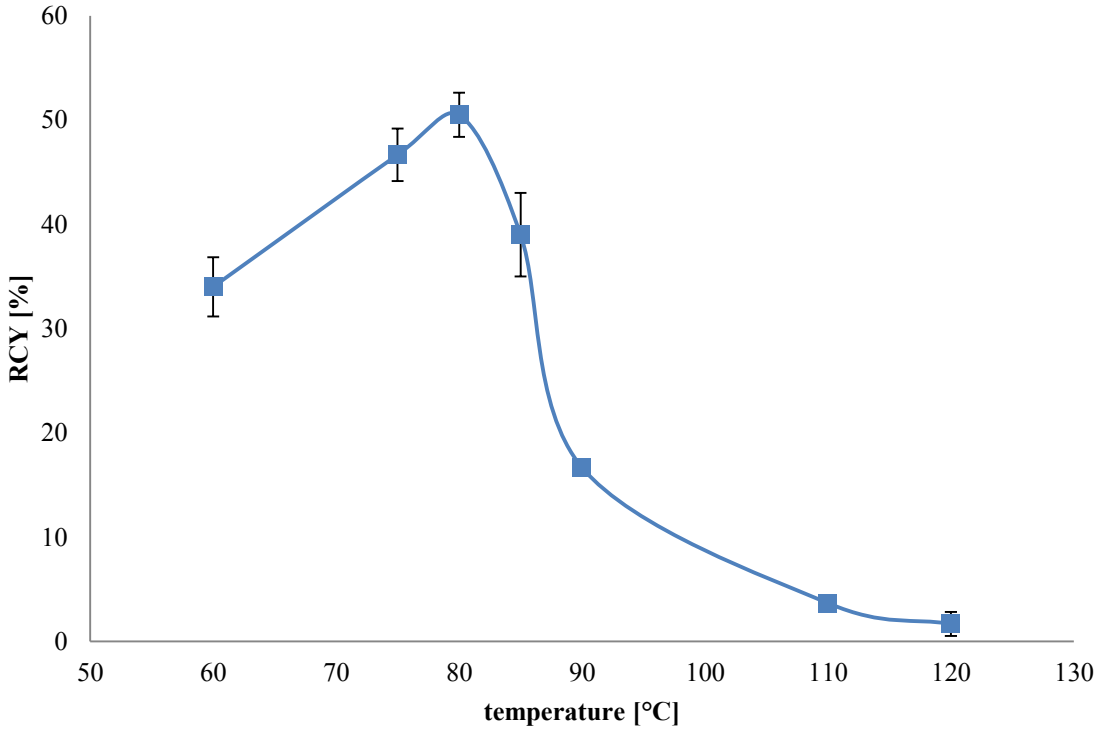


Figure 11 Temperature dependence of the isotopic exchange of **68** on the RCY of **[^{18}F]68**.

Due to the fact that the isotopic exchange on **68** strongly depends on the temperature of the reaction, it was additionally studied whether a reaction time of 15 min is useful or whether decomposition is already occurring after this time. For those investigations, the isotopic exchange was performed at the optimum temperature of 80 °C and samples of the reaction mixture were taken and analyzed by radio HPLC after 5, 10, 15, 20, 25, 30 and 45 min. The obtained yields over time are shown in Figure 12. It showed that the maximum RCY is already reached after 10 min under the studied conditions. Furthermore, a plateau of the RCY was observed for reaction times between 10 and 20 min. Decomposition of the labeled intermediate **[^{18}F]68** started at reaction times longer than 20 min. The decomposition, however, was slow but led to a total loss of **[^{18}F]68** of about 13 % during 45 min. According to these results, the initially used reaction time of 15 min was optimal, since it is placed in the middle of the plateau. After this time, it can be assumed that the isotopic exchange is completed but decomposition of the product has not started at this time point.

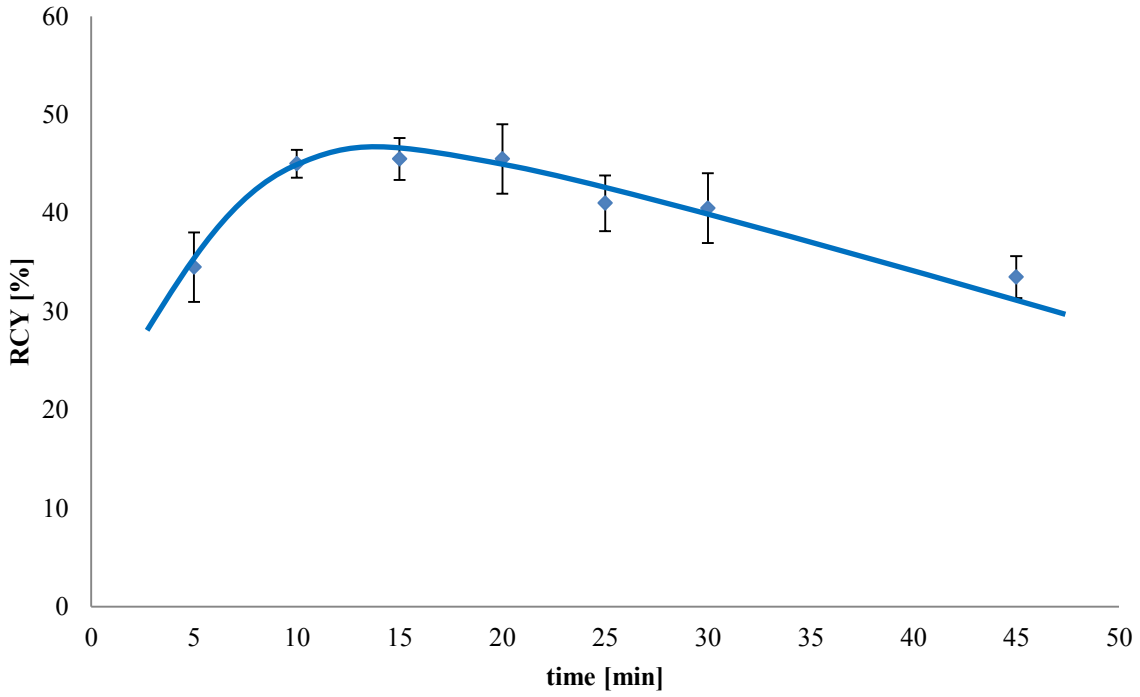


Figure 12 Optimization of the isotopic exchange with respect to reaction time.

Moreover, it was investigated if a microwave assisted setup, which gave excellent RCY with the 1-benzyl-fluoro-1*H*-indole-carbaldehydes (see chapter 3.2.1), is also suitable for the isotopic exchange on the precursor **68** to obtain higher RCY.

Therefore, the reaction mixture was irradiated with 30 – 80 W microwaves for 1 min. Afterwards the reaction mixture was cooled to room temperature and analyzed by radio-

HPLC. The results obtained with the microwave assisted setup are shown in Figure 13. It was found that radiolabeling is possible using microwave heating with low microwave energies of 30 to 40 W. At energies of 60 W decomposition of the precursor was observed which was probably due to temperatures above 90 °C in the reactor and the high sensitivity of the precursor towards elevated temperatures. The best results were obtained when irradiation was done with 40 W which yielded **[^{18}F]68** in a RCY of about 40 %. However, this was ca. 10 % less than under conventional heating. Furthermore, the variance of the RCY obtained under microwave heating was higher than under conventional heating e.g. at 40 W, RCY between 25 % and 44 % were obtained. This is in contrast to previous results from the literature where the RCY was described as more stable with microwave heating than under conventional heating.[173] The most positive result of the microwave assisted conditions was that it was again possible to reduce the reaction time from 15 to 1 min.

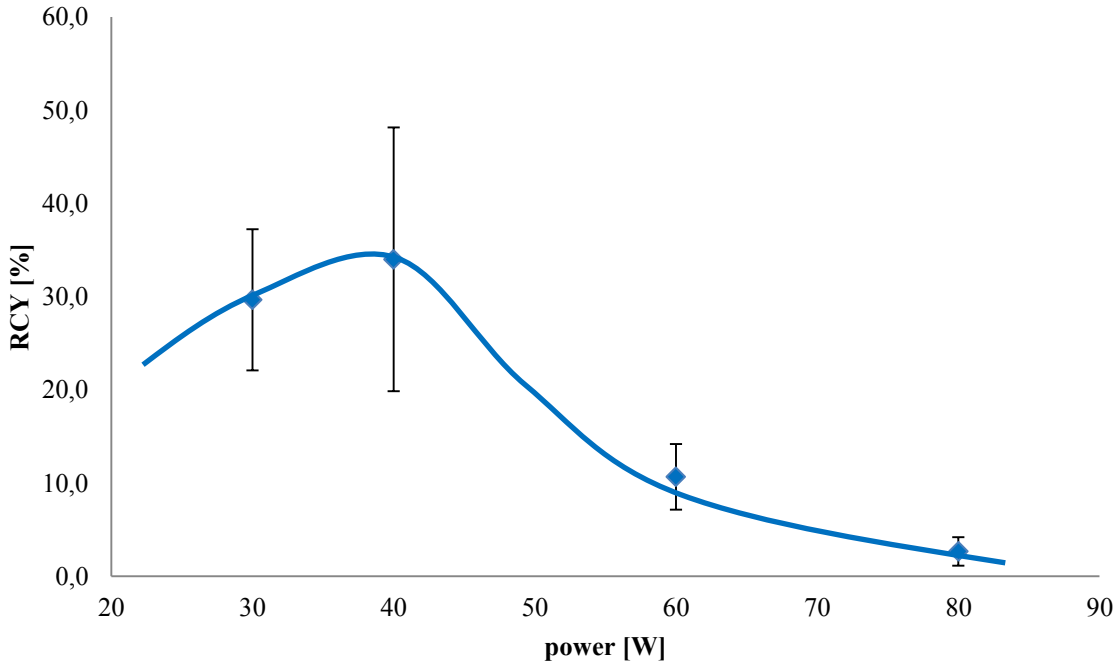


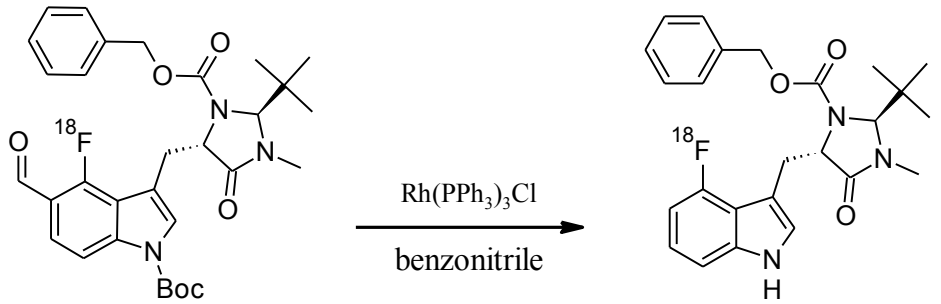
Figure 13 Isotopic ^{18}F-for-^{19}F exchange on the precursor 68 under microwave heating.

In summary, radiofluorination by isotopic exchange on the precursor **68** was successful using $TBAHCO_3$ as anion activator and DMF as solvent. The desired product **[^{18}F]68** could be obtained in about 50 % RCY under conventional heating for 15 min at 80 °C and in about 40 % under microwave assisted heating with 40 W microwaves within 1 min. However, the obtained RCY were much lower compared to those obtained for 1-benzyl-fluoro-1*H*-indolecarbaldehydes, but higher than those obtained for 1-Boc-4-fluoro-1*H*-indole-5-carbaldehyde (**2g**). Further, a second diastereomer that occurred during the isotopic exchange

of **43** could not be detected with **68** and was probably due to the lower temperatures during the isotopic exchange reaction.

3.7.2 Reductive Decarbonylation of [^{18}F]68

The second step in the synthetic pathway of the radiosynthesis of L-4-[^{18}F]fluorotryptophan (**[^{18}F]70**) was the removal of the formyl group that was previously attached for the activation of the isotopic exchange. The removal was again carried out with Wilkinson's catalyst ($Rh(PPh_3)_3Cl$).[173] The reaction itself was performed under the conditions that gave the best RCY with the 1-benzyl-[^{18}F]fluoro-1*H*-indolecarbaldehydes **[^{18}F]1e**, **[^{18}F]2e**, **[^{18}F]3e** and **[^{18}F]4e**. Thus, all reactions were carried out in benzonitrile. The first attempt was made with 3 equivalents of $Rh(PPh_3)_3Cl$. However, it was not possible to obtain the decarbonylated intermediate **[^{18}F]69** without the loss of the Boc-group that was attached to the indole nitrogen. This was not a problem since it had to be removed in the hydrolysis step anyway (Scheme 72).



[^{18}F]68 [^{18}F]69a

Scheme 72 Loss of the Boc group during the reductive decarbonylation on **[^{18}F]68**.

When the reaction was examined with 3 equivalents of $Rh(PPh_3)_3Cl$ under conventional heating to 150 °C for 20 min the decarbonylated and deprotected **[^{18}F]69a** was obtained in a RCY of about 45 % while the formation of a polar side product was also observed but could not be identified. Therefore, the amount of $Rh(PPh_3)_3Cl$ was risen in order to get a higher RCY. Increasing the amount of $Rh(PPh_3)_3Cl$ to 4 equivalents decreased the RCY to only 35 %, while the formation of the side product was increasing. Hence, the amount of $Rh(PPh_3)_3Cl$ was reduced to 2 equivalents which resulted in a reduced RCY of about 15 %

and only minor amounts of the polar side product (< 10 %) but a total conversion of only about 22 %. Due to these results the initially used amount of $Rh(PPh_3)_3Cl$ resulted in the highest RCY with conventional heating.

A microwave assisted setup was also studied for the reductive decarbonylation in order to increase the obtained RCY or reduce the time needed for the reaction. First, the amount of catalyst that worked best for the reductive decarbonylation of **[^{18}F]68** under conventional heating, which were 3 equivalents, was also used and the solution was irradiated with 100 W microwaves for 2 min. This microwave energy and reaction time were chosen, since good results were obtained before with the 1-benzyl-fluoro-1*H*-indolecarbaldehydes **[^{18}F]1e**, **[^{18}F]2e**, **[^{18}F]3e** and **[^{18}F]4e**. Under those conditions, a RCY of ca. 75 % was achieved which was significantly higher than under conventional heating. Reducing the amount of catalyst to 1 equivalent led to a decrease of the RCY to about 58 %. When the amount of $Rh(PPh_3)_3Cl$ was again risen to 2 equivalents the RCY was also increasing to ca. 69 %. Further increasing of the amount of $Rh(PPh_3)_3Cl$ to 4 equivalents did, however, not result in a significantly higher RCY. The formation of the same polar side product detected under conventional heating was always observed. The results are summarized in Table 8.

Table 8 Results of the reductive decarbonylation of **[^{18}F]68**; CH: conventional heating for 20 min, MH: microwave heating for 2 min.

Conditions	eq. $Rh(PPh_3)_3Cl$	Time [min]	Conversion [%]	RCY [%]
CH, 150 °C	2	20	22 ± 8	15 ± 6
CH, 150 °C	3	20	65 ± 9	45 ± 8
CH, 150 °C	4	20	45 ± 6	35 ± 3
MH, 100 W	1	2	> 99	58 ± 11
MH, 100 W	2	2	> 99	69 ± 9
MH, 100 W	3	2	> 99	75 ± 7
MH, 100 W	4	2	> 99	78 ± 6

In order to reduce the amount of the undesired side product that is formed during reductive decarbonylation, the reaction was tested with an irradiation time of 60 s and 3 equivalents of

Wilkinson's catalyst. This led to surprising results: The formation of four products, the starting material **[^{18}F]68**, the decarbonylated but still Boc protected intermediate **[^{18}F]69**, the decarbonylated and deprotected intermediate **[^{18}F]69a** and the unknown side product, always formed during reductive decarbonylation. This shows that the reaction time does not correlate with the unknown side product and that this has to be subject to something else. Furthermore, those results were interesting since they indicate that the irradiation time of 2 min is necessary to guarantee a complete conversion during the reaction.

In summary, the reductive decarbonylation could be performed under both conventional and microwave assisted heating. However, when microwave heating was applied, the obtained RCY was significantly higher than under conventional heating. The reaction times could thus be reduced from 20 min to 2 min, which is of great advantage in radiochemistry. The decarbonylated products were purified as described in the Experimental part (see chapter 4.7.4).

3.7.3 Hydrolysis of protecting groups

The last step in the radiosynthesis of L-4-[^{18}F]fluorotryptophan was the hydrolysis of the Seebach auxiliary which masks the amino acid function. Due to the fact that the Boc-group was already removed during reductive decarbonylation the focus for the conditions of the hydrolysis was adapted to Seebach's auxiliary. The amide bond in this group is quite stable and its cleavage requires rather harsh reaction conditions.

In the past this was done using concentrated HBr or HI and temperatures as high as 150 °C and 200 °C, respectively.[168],[199] In a work published in 2011 concentrated HCl and heating to 150 °C for 30 min was used for the hydrolysis of the Boc-protected derivative of Seebach's auxiliary.[173] Here, these conditions were adapted giving a RCY of only ca. 34 % which was unexpected, since those types of reactions were described as quantitative in the past. Additionally, a side product was formed in about 13 % of the total activity that could not be characterized (see Figure 14). Due to the possibility that the side product might be subject to incomplete hydrolysis of the protecting groups it was studied if longer reaction times would provide a higher RCY. Therefore, the hydrolysis was performed for 2 h and samples were taken every 30 min. It proved that the unknown peak is not a result of incomplete hydrolysis, since the ratio of L-4-[^{18}F]fluorotryptophan and of this peak stayed the same over the whole

reaction time. Hence, it was assumed that decomposition of the temperature sensitive starting material might be the reason for the occurrence of the side product.

Furthermore, changes in reaction temperatures could provide a better RCY for this reaction and those were varied from 110 to 160 °C. While at temperatures lower than 130 °C the RCY fell down due to incomplete hydrolysis, a reaction temperature of 160 °C resulted in complete decomposition of the starting material. In total the conditions first tested (150 °C, 30 min) gave the best results and were used for further experiments.

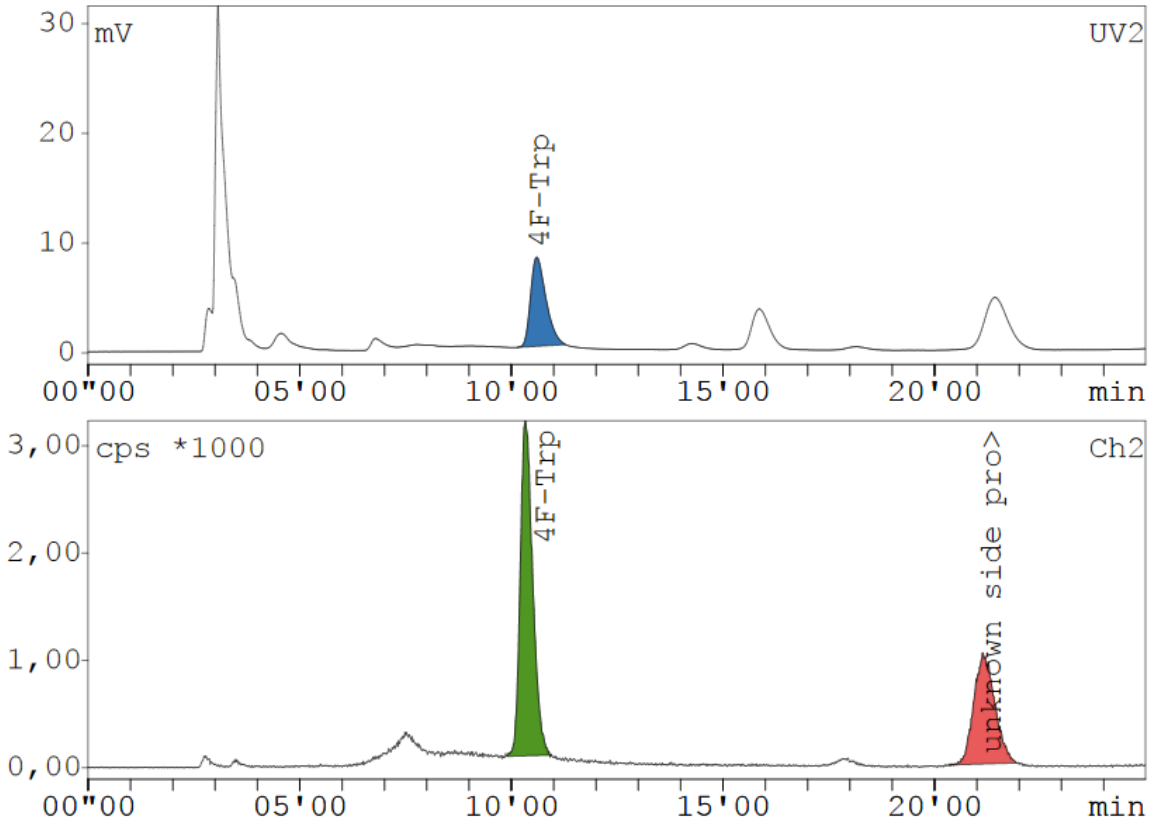


Figure 14 HPLC-analysis of L-4-[^{18}F]fluorotryptophan (**[^{18}F]70**) and an undesired side product which is formed during hydrolysis of **[^{18}F]69a**.

In summary, the radiosynthesis of L-4-[^{18}F]fluorotryptophan could be successfully accomplished in three steps with an overall RCY of about 13 % and an enantiomeric purity of > 99 %. Figure 15 depicts a comparison between the HPLC analysis of a standard sample of L-4-fluorotryptophan prepared as described by Konas et al.[160] and the radiofluorinated L-4-[^{18}F]fluorotryptophan which was previously purified by semi-preparative HPLC.

The moderate total RCY of about 13 % is mainly due to the hydrolysis which gave only 34 % RCY. Thus, it can probably be increased during further optimization of this reaction step or by using a protecting group for the amino acid function that can be hydrolyzed under milder conditions and thereby avoiding decomposition of the intermediate **[^{18}F]69a**.

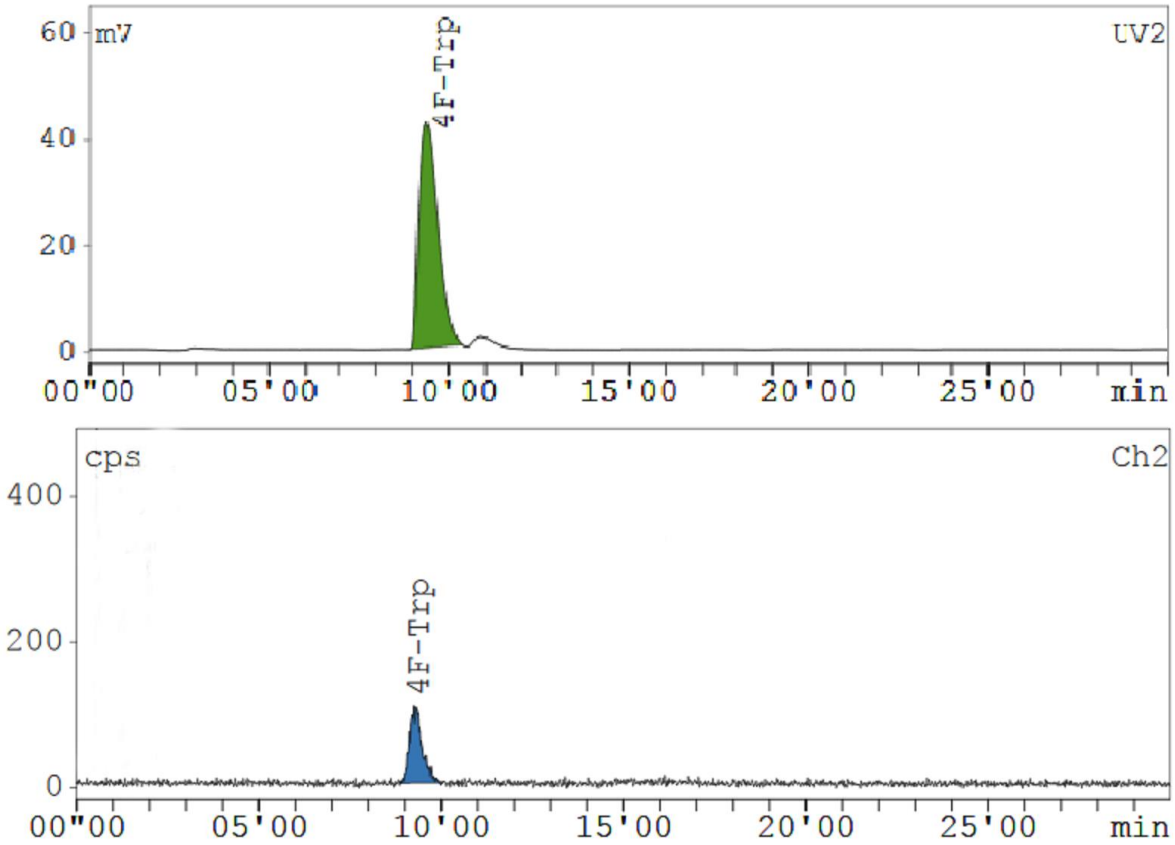


Figure 15 HPLC analysis on a Supelco chirobiotic™ T coloumn (250x4.6 mm) of a standard sample of L-4-fluorotryptophan and the previously purified final product **[^{18}F]70** after hydrolysis (**[^{18}F]70** = 4FTrp).

3.7.4 Specific activity

The specific activity obtained in isotopic exchange reactions is a function of both, the amount of precursor and the starting ^{18}F-activity. The amount of carrier is determined by the quantity of precursor employed, in this case ca. 9 µmol. Under manual experimental conditions used here with a low starting activity of typically 250 MBq the specific activity was always > 70 MBq/mmol which is in the area of specific activities that can be obtained under electrophilic fluorination conditions. But it can be expected that the specific activity will increase significantly with higher starting activities and will thereby be much higher than those obtained with electrophilic fluorination.

3.8 Summary of the obtained results

In this work, a simple three-step radiosynthesis of L-4-[^{18}F]fluorotryptophan was developed. The used precursor was synthesized in an eleven-step linear synthesis in an overall yield of 8 %. The substitution pattern of the fluorine and the formyl group for the precursor **68** was chosen with respect to the results that were obtained prior to its synthesis in order to determine the optimum substitution pattern. Furthermore, the precursor **43**, which had the fluorine in the undesirable 6-position, confirmed the results obtained for the fluoro-1*H*-indole-carbaldehydes regarding the isotopic exchange.

However, further investigations are necessary in order to increase the yield for the radiosynthesis of L-4-[^{18}F]fluorotryptophan, especially the hydrolysis. This can probably be achieved through a protection group on the amino acid function which can be cleaved under less aggressive conditions. A promising attempt for this is the protection of the carboxylic acid with an ester and the protection of the amine with a trityl group that can be hydrolyzed under slightly acidic conditions at room temperature. It is also desirable to increase the stability of the precursor towards elevated temperatures, since this was also an issue during the ^{18}F-for-^{19}F isotopic exchange and might also result in higher RCY during this reaction due to the fact that higher labeling temperatures become possible.

4. Experimental

4.1 General techniques

All reactions sensitive to humidity were carried out under an atmosphere of argon. The reaction flasks were dried for 24 h at 100 °C and equipped with a septum and a balloon filled with argon. All liquids sensitive to humidity were transferred into the reaction flask through syringes. All reaction mixtures were magnetically stirred.

4.1.1 Spectrometric devices

^{1}H, ^{13}C and ^{19}F spectra were recorded on a Varian Inova 400 MHz (ZEA-3) or a Bruker DPX Avance (INM-5) spectrometer using $CDCl_3$ or d_6-DMSO as solvents. All shifts are given in ppm using the solvent residual signals as reference. HRMS spectra were obtained on a FTICR 'LTQ FT Ultra (Thermo Fisher Scientific, Germany) by the ZEA-3. Elemental analyses were obtained on a Vario EL cube (ZEA-3).

4.1.2 Microwave device

Reactions under microwave heating were performed using a CEM Discover (Matthews, USA) single-mode microwave reactor system in the energy range from 30 to 200 W.

4.1.3 Preparative chromatography and analytic thin layer chromatography

Flash chromatography was performed on silica gel (Merck 60 mesh for flash chromatography, Germany) following the procedure proposed by Still[200] or via automated flash chromatography using a Reveleris Flash Chromatography system (Grace, USA) on commercially available Flash Grace Reveleris™ cartridges (Grace, USA).

Thin layer chromatography (TLC) was performed on precoated plates of silica gel 60 F_{254} (Merck, Germany). The compounds were detected by UV absorption at 254 nm.

4.1.4 Radioanalytik procedures

The analysis of the radiofluorinated products was performed by either radio thin layer chromatography (radio-TLC) or radio high performance liquid chromatography (radio-HPLC).

Radio-thin layer chromatography

Radio-TLC for the analysis of the ^{18}F-labeled products and intermediates was done in order to determine the radiochemical yield (RCY). The measurements were performed on precoated silica plates of silica gel 60 F_{254} (Merck, Germany). The reaction mixtures were diluted with an appropriate solvent (Et_2O or MeCN) and samples of about 2 µL were applied on the TLC plates. Those were developed in an appropriate solvent mixture of petroleum ether (PE) and ethyl acetate (EA). The identification of unreacted [^{18}F]fluoride was simple since under the conditions used for the elution it remained at the origin of the TLC plate. The distribution of the radioactivity on the TLC plate was detected using an Instant Imager™ (Packard Instruments). The RCY was calculated by dividing the activity of the spot of compound of interest by the total activity on the TLC-plate, multiplied by 100 (Equation 1). Table 9 shows the corresponding R_f-values and eluent mixtures of the labeled compounds analyzed by radio-TLC.

$$RCY\ [\%] = \frac{A_{Compound}}{A_{total}} \times\ 100$$

Equation 1 Determination of the radiochemical yield by radio-TLC.

Table 9 R_f-values and eluent systems for the compounds determined by radio TLC..

Compound	Eluent system (PE/EA)	R_f-value
[^{18}F]1d, [^{18}F]2d, [^{18}F]3d	3:2	0.63
[^{18}F]1e, [^{18}F]2e, [^{18}F]3e	4:1	0.65
[^{18}F]1f	4:1	0.63
[^{18}F]1g, [^{18}F]2g	4:1	0.69
[^{18}F]1h	4:1	0.59
[^{18}F]43	3:2	0.62
[^{18}F]68	7:3	0.74
[^{18}F]69	7:3	0,24

Radio-high performance liquid chromatography

Radio-HPLC separations were performed using a Knauer pump (Knauer, Germany), a Knauer K-2500 UV/VIS detector (Knauer, Germany) and two manual Rheodyne injectors (20 µL loop). The first loop was located before the HPLC-column and the second one behind it. Detection of radioactivity was accomplished with a NaI(Tl) well-type scintillation detector (EG&G Ortec; model 276 Photomultiplier Base, Ametek, USA) with an ACE Mate Amplifier and BIAS supply (Ortec, USA). Data acquisition and interpretation was done with Gina software (Raytest, Germany).

Radioactive samples were injected into the column using the first injector. After elution of all compounds of interest from the column, three aliquots of the sample were injected into the second injector. The RCY of the product was determined using equation 2 where $A_{product}$ is the activity of the desired product peak and A_{total} is the activity determined by injection into the second injector. The term $e^{-\lambda t}$ is used for decay correction during the HPLC run, where "λ" is the decay constant for fluorine-18 and "t" is the time difference between the retention times of the product and the aliquots.

$$RCY\ [\%] = \frac{A_{Product} \times 100}{A_{total} \times e^{-\lambda t}}$$

Equation 2 Determination of the radiochemical yield by radio HPLC.

HPLC-Systems

System A. Analytic HPLC of the ^{18}F-labeled indole derivatives was performed with a reverse-phase Kromasil 5 C18 column (250x4 mm; CS Chromatographieservice GmbH, Germany). Elution was performed at a constant flow rate of 1 $mL \cdot min^{-1}$ with a acetonitrile–water mixture (65 : 35).

System B. Preparative HPLC was carried out with a Synergi 4m Hydro-RP 80A column (250–10 mm; Phenomenex, Germany). The mobile phase was aqueous ethanol (10 %), and the flow rate was 3 $mL \cdot min^{-1}$.

System C. The enantiomeric purity of the radiolabeled compounds was determined by HPLC using a Supelco chirobiotic™ T column (250x4.6 mm; Sigma-Aldrich, Germany). Elution was performed at a constant flow rate of 1 $mL \cdot min^{-1}$ with a methanol-water mixture (30:70).

System D. Analytical HPLC was carried out with a Synergi 4m Hydro-RP 80A column (250–4 mm; Phenomenex, Germany). The mobile phase was aqueous ethanol (10 %), and the flow rate was 1 $mL \cdot min^{-1}$.

Table 10 k' values of the probes analyzed by radio-HPLC.

Compound	System	k’
[^{18}F]1e	A	5.18
[^{18}F]2e	A	5.26
[^{18}F]3e	A	5.38
[^{18}F]4e	A	6.60
[^{18}F]5	A	7.49
[^{18}F]6	A	7.84
[^{18}F]7	A	8.32
[^{18}F]43	A	9.94/10.91 (L + D)
[^{18}F]68	A	14.68
[^{18}F]69	A	5.18
[^{18}F]70	B	3.44
[^{18}F]70	C	6.18
[^{18}F]70	D	2.99

4.1.5 Reagents and solvents

The dry solvents, dichloromethane, tetrahydrofurane, dioxane, methanol, *N,N*-dimethylformamide, diethyl ether acetone, benzonitrile, dimethyl sulfoxide, acetonitrile, benzene, toluene and anisole, were purchased from Sigma-Aldrich, Germany. The solvents used for extraction and chromatography, diethyl ether, petroleum ether, hexanes, acetonitrile

(HPLC grade), ethyl acetate, ethanol, methanol, dichloromethane, were obtained from Merck, Germany. All solvents were used without further purification or drying.

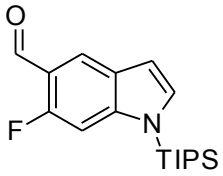
(S)-Boc-BMI, *(S)*-Z-BMI, 1,2-ethanedithiol, 1-bromo-4-fluoro-2-nitrobenzene (**4a**), 2-amino-6-fluorobenzoic acid (**46**), 3-fluoroaniline (**19**), 4-bromo-2-fluorobenzaldehyde (**24**), 6-fluoroindole (**1a**), 7-fluoroindole (**3a**), aluminum chloride, ammonium chloride, ammonium formate, benzaldehyde, benzyl bromide, boron trifluoride diethyl etherate, cesium carbonate, chloroethylformate, diiodoethane, diisobutylaluminium hydride, di-tert-butyl dicarbonate, ethyl diazoacetate, iodomethane, isopropyl magnesium bromide, isopropyl magnesium chloride, lithium aluminum hydride, lithium chloride, manganese dioxide (activated), *N,N,N',N'',N''*-pentamethyl-diethylenetriamine, *N,N*-dimethylaminopyridine, *n*-butyllithium, palladium on activated charcoal, palladium(II)acetate, phosphorous tribromide, potassium carbonate, potassium iodide, *sec*-butyllithium, silver sulfate, sodium borohydride, sodium carbonate, sodium chloride, sodium cyanoborohydride, sodium hydride, sodium hydroxide, sodium sulfate, sodium thiosulfate, *tert*-butylcarbamate, tetrabutylammonium fluoride, tosyl chloride, triethylamine, trifluoroacetic acid, trimethylsilyl chloride, triphenylphosphine, triphosgene, triisopropylsilyl chloride, vinyl magnesium bromide, Wilkinson catalyst, Xphos and zinc chloride were all acquired from Sigma-Aldrich, Germany.

4-Fluoroindole, D,L-4-fluorotryptophan, D,L-6-fluorotryptophan and phosphorous oxy-chloride were purchased from Chempur, Germany, while acetic acid, hydrobromic acid and hydrochloric acid were acquired from Merck, Germany.

IBX was prepared as described by Frigerio et al. [201]. Dess-Martin periodan was synthesized as described by Dess and Martin [202]. 1-Trisiopropylsilyl-4-fluoro-1*H*-indole (**2b**), 1-trisiopropylsilyl-6-fluoro-1*H*-indole (**1b**), 1-trisiopropylsilyl-7-fluoro-1*H*-indole (**3b**)and 7-bromo-4-fluoro-1*H*-indole (**4b**) were prepared as described by Schosser et al. [69].

4.2 Synthesis of fluoro-1*H*-indole-carbaldehyde precursors

1-Triisopropylsilyl-6-fluoro-1*H*-indole-5-carbaldehyde (1c),
according to Schlosser et al.[69]

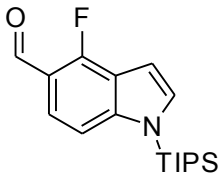


A solution of 6-fluoro-1-(triisopropylsilyl)indole (4.0 g, 14 mmol) in 30 mL tetrahydrofuran was cooled to –78 °C, and a 1.4 M solution of sec-butyllithium in hexanes (14 mmol) was added dropwise. The resulting mixture was stirred for 2 h at -78 °C before 5 mL dimethylformamide were added slowly. The reaction was allowed to warm up to room temperature and stirred for 2 more hours. Water was added and the aqueous phase was extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4, filtered and the solvents were evaporated *in vacuo*. The crude residue was purified via flash chromatography (2 % EtOAc/PE, R_f = 0,30) giving 2.5 g (7.8 mmol, 64%) of the desired product as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ 10.38 (s, 1H), 8.17 (d, J = 7.0 Hz, 1H), 7.32 (d, J = 3.3 Hz, 1H), 7.24 (dd, J = 12.6 Hz, J = 0.7, 1H), 6.74 (dd, J = 2.4 Hz, J = 0.9 Hz, 1H), 1.72 (hept, J = Hz, 3H), 1.19 (d, J = 7.4 Hz, 18H); ^{13}C-NMR ($CDCl_3$) 187.11 (J = 6.4 Hz), 161.13 (J = 246.6), 144.77 (J = 12.1 Hz), 133.44 (J = 3.1 Hz), 128.17 (J = 1.1 Hz), 121.68 (J = 4.0 Hz), 118.37 (J = 10.9 Hz), 106.52, 100.38 (J = 25.2 Hz), 17.99, 12.68; ^{19}F-NMR ($CDCl_3$) δ -130.1

HRMS: $C_{18}H_{26}FNOSi$ $[M+H]^+$ calcd.. 320.1840 found 320.1839

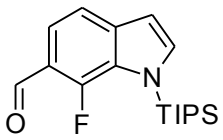
1-Triisopropylsilyl-4-fluoro-1*H*-indol-5-carbaldehyd (2c) according to Schlosser et al.[69]



Analogously prepared as the aldehyde **1c** while starting from 4-fluoro-1-(triisopropylsilyl)indole (**2b**) giving the desired product as a colorless solid in 66 % yield.

^{1}H-NMR (DMSO) δ 10.26 (s, 1H), 7.51-7.48 (m, 3H), 6.85 (d, J = 3.2 Hz), 1.73 (hept, 3H, J = 7.6 Hz), 1.02 (d, 18H, J = 7.6 Hz); ^{13}C-NMR (DMSO) δ 187.27 (J = 5.8 Hz), 158.86 (J = 259.3 Hz), 147.31 (J = 11.5 Hz), 134.24, 120.94, 119.68 (J = 19 Hz), 115.77 (J = 5.3 Hz), 111.53, 102.29, 18.13, 12.23; ^{19}F-NMR (DMSO) δ -128,78; HRMS: $C_{18}H_{26}FNOSi$ $[M+H]^+$ calcd.. 320.1840 found 320.1842; E.A. calcd. C: 67.67 H: 8.20 N: 4.38 found C: 67.68 H: 8.25 N: 4.35

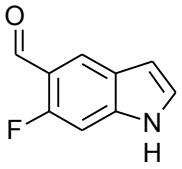
1-Triisopropylsilyl-7-fluoro-1*H*-indol-5-carbaldehyd (3c) according to Schlosser et al.[69]



Analogously prepared as the aldehyde **1c** but starting from 7-fluoro-1-(triisopropylsilyl)indole (**3b**) giving the desired product as a colorless solid in 63 % yield.

^{1}H-NMR (DMSO) δ 10.32 (s, 1H), 7.71 (d, 1H, J = 2.6 Hz), 7.52 (dd, 1H, J = 8.2 Hz, J = 12.6 Hz), 7.51 (d, 1H, J = 7.1 Hz), 6.82 (s, 1H), 1.69 (sept, 3H, J = 7.2 Hz), 1.07 (d, 18H, J = 7.5 Hz); ^{13}C-NMR (DMSO) δ 187.51 (J = 8.3 Hz), 153.08 (J = 257.1 Hz), 140.90 (J = 7.6 Hz), 138.37, 126.92 (J = 9.4 Hz), 119.6, 117.36, 106.80, 18.25, 13.14 (J = 5.2 Hz); ^{19}F-NMR (DMSO) δ -133.15; HRMS: $C_{18}H_{26}FNOSi$ $[M+H]^+$ calcd. 320.1840 found 320.1840; E.A.: calcd. C: 67.67 H: 8.20 N: 4.38 found C: 67.42 H: 8.24 N: 4.35

6-Fluoro-1*H*-indole-5-carbaldehyde (1d) according to Schlosser et al.[69]

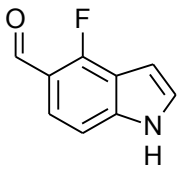


4.1 mL of a 1.0 M solution of TBAF in THF were added to a solution of 1.3 g (4.0 mmol) of 1-triisopropylsilyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1c**) in 5 mL THF. The reaction mixture was stirred for 5 min at room temperature, quenched by the addition of water and extracted repeatedly with Et_2O. The combined organic layers were dried over Na_2SO_4 filtered and the solvent was removed *in vacuo*. Purification by column chromatography (PE/EA = 3:2) gave 560 mg (3.4 mmol, 86 %) of the desired 6-fluoro-1*H*-indole-5-carbaldehyde (**1d**) as a colorless solid.

M.p.: decomposition at 125 – 128 °C

^{1}H-NMR (DMSO) δ 11.60 (s, 1H), 10.16 (s, 1H), 8.06 (d, 1H, J = 6.8 Hz), 7.47 (t, 1H, J = 3 Hz), 7.28 (d, 1H, J = 12 Hz), 6.61 (s, 1H); ^{13}C-NMR (DMSO) δ 188.18 (J = 4.9 Hz), 160.16 (J = 245.7 Hz), 139.72 (J = 13.4 Hz), 128.83 (J = 2.7 Hz), 124.91, 123.22 (J = 3.8 Hz), 117.88 (J = 10.7 Hz), 103.728, 98.43 (J = 24.8 Hz); ^{19}F-NMR (DMSO) δ -130.24; HRMS: C_9H_6FNO $[M+H]^+$ calcd. 164.0506 found 164.0506; E.A.: calcd. C: 66.26 H: 3.71 N: 8.59 found C: 65.80 H: 3.75 N: 8.55

4-Fluoro-1*H*-indole-5-carbaldehyde (2d) according to Schlosser et al.[69]

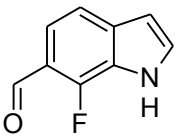


Analogously prepared as compound **1d** while starting from 1-triisopropylsilyl-4-fluoro-1*H*-indol-5-carbaldehyd (**2c**); colorless solid; 89%

^{1}H-NMR (DMSO) δ 11.89 (s, 1H), 10.27 (s, 1H), 7.49 (t, 2H, J = 6.8 Hz),

7.33 (d, 1H, J = 8.4 Hz), 6.68 (s, 1H); ^{13}C-NMR (DMSO) δ 187.23 (J = 6.9 Hz), 159.50 (J = 259.5 Hz), 142.84 (J = 13 Hz), 128.18, 120.45, 116.21 (J = 20.6 Hz), 115.15 (J = 5.4 Hz), 109.38 (J = 3 Hz), 99.33; ^{19}F-NMR (DMSO) δ -129,01; HRMS: C_9H_6FNO $[M+H]^+$ calcd. 164.0506 found 164.0506; E.A.: calcd. C: 66.26 H: 3.71 N: 8.59 found C: 65.76 H: 3.73 N: 8.54

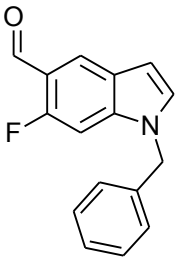
7-Fluoro-1*H*-indole-6-carbaldehyde (3d) according to Schlosser et al.[69]



Analogously prepared as aldehyde **1d** but starting from 1-triisopropylsilyl-7-fluoro-1*H*-indol-5-carbaldehyd (**3c**) giving the desired product as a colorless solid in 88 % yield.

^{1}H-NMR (DMSO) δ 10.28 (s, 1H), 7.67-7.34 (m, 2H), 7.41-7.37 (m, 1H), 6.59 (s, 1H); ^{13}C-NMR (DMSO) δ 187.32 (J = 6.8 Hz), 152.7 (J = 258.9 Hz), 137.00 (J = 7.8 Hz), 131.63, 123.24 (J = 11.24 Hz), 117.92, 116.97 (J = 2.9 Hz), 116.46 (J = 3.4 Hz), 103.66 (J = 1.2 Hz); ^{19}F-NMR (DMSO) δ -140.48; HRMS: C_9H_6FNO $[M+H]^+$ calcd. 164.0506 found 164.0505; E.A.: calcd. C: 66.26 H: 3.71 N: 8.59 found C: 65.89 H: 3.75 N: 8.62

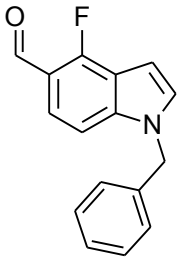
1-Benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (1e)



A solution of 270 mg (1.7 mmol) of 6-fluoro-1*H*-indole-5-carbaldehyde (**1d**) in 5 mL DMF was cooled to 0 °C, and 100 mg (2.5 mmol) of a 60% dispersion of NaH in mineral oil were added. The resulting solution was stirred for 20 min at 0 °C before 296 µL (2.5 mmol) benzyl bromide were added. Then, the reaction mixture was warmed to room temperature and stirred for 12 h. Water was added, followed by repeated extraction with Et_2O. The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. Purification by column chromatography gave 371 mg (1.46 mmol, 86 %) of 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**) as a colorless solid.

^{1}H-NMR (DMSO) δ 10.17 (s, 1H), 8.08 (d, 1H, J = 6.8 Hz), 7.63 (d, 1H, J = 3.1 Hz), 7.54 (d, 1H, J = 12.5 Hz), 7.32-7.22 (m, 5H), 5.43 (s, 2H); ^{13}C-NMR (DMSO) δ 188.09 (J = 5.3 Hz), 160.31 (J = 246.4 Hz), 139.58 (J = 13 Hz), 137.86, 132.67 (J = 3.1 Hz), 129.12, 128.06, 127,65, 125.32, 123.59 (J = 3.8 Hz), 118.04 (J = 10.7 Hz), 104.07, 97.68 (J = 26 Hz), 49.77; ^{19}F-NMR (DMSO) δ -128.90; HRMS: $C_{16}H_{12}FNO$ $[M+H]^+$ calcd. 254.0976 found 254.0975; E.A.: calcd. C: 75.88 H: 4.78 N: 5.53 found C: 75.27 H: 4.86 N: 5.27

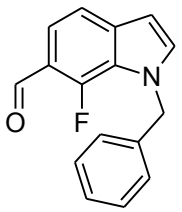
1-Benzyl-4-fluoro-1*H*-indole-5-carbaldehyd (2e)



Analogously prepared as the aldehyde **1e** while starting from 4-fluoro-1*H*-indole-6-carbaldehyde (**2d**) giving the desired product as a colorless solid in 88 % yield.

^{1}H-NMR (DMSO) δ 10.26 (s, 1H), 7.68 (d, 1H, J = 3.2 Hz), 7.49 (dd, 1H, J = 8.8 Hz, J = 8.4 Hz), 7.30-7.20 (m, 5H), 6.75 (d, 1H, J = 2.8 Hz), 5.47 (s, 2H); ^{13}C-NMR (DMSO) δ 187.24 (J = 6.1 Hz), 159.24 (J = 260.1 Hz), 142.27 (J = 13 Hz), 137.81, 132.00, 129.01 (J = 20.6 Hz), 128.11 (J = 7.6 Hz), 127.50, 116.76 (J = 20.6 Hz), 115.40 (J = 5.3 Hz), 108.21 (J = 3.1 Hz), 99.45, 49.94; ^{19}F-NMR (DMSO) δ -128.90; HRMS: $C_{16}H_{12}FNO$ $[M+H]^+$ calcd. 254.0976 found 254.0975; E.A.: calcd. C: 75.88 H: 4.78 N: 5.53 found C: 75.27 H: 4.86 N: 5.27

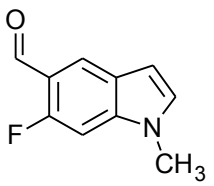
1-Benzyl-7-fluoro-1*H*-indole-6-carbaldehyd (3e)



Analogously prepared as the aldehyde **1e**, but starting from 7-fluoro-1*H*-indole-6-carbaldehyde (**3d**) giving the desired product as a colorless solid in 82 % yield.

^{1}H-NMR ($CDCl_3$) δ 10.36 (s, 1H), 7.52 (dd, 1H, J = 5.9 Hz, J = 2.1 Hz), 7.39 (d, 1H, 8.3 Hz), 7.33-7.24 (m, 4H), 7.13 (d, 2H, J = 7.0 Hz), 6.58 (s, 1H), 5.51 (s, 2H); ^{13}C-NMR ($CDCl_3$) δ 187.03 (J = 8.7 Hz), 153.67 (J = 258.8 Hz), 137.76 (J = 7.4 Hz), 137,36, 133.88, 128.90, 127.98, 126.70, 122.98 (J = 8.1 Hz), 118.44, 117.61 (J = 4.5 Hz), 117.13 (J = 3.3 Hz), 103.67 (J = 1.2 Hz), 52.65 (J = 5.6 Hz); ^{19}F-NMR ($CDCl_3$) δ -148.05; HRMS: $C_{16}H_{12}FNO$ $[M+H]^+$ calcd. 254.0976 found 254.0976; E.A.: calcd. C: 75.88 H: 4.78 N: 5.53 found C: 75.57 H: 4.80 N: 5.49

1-Methyl-6-fluoro-1*H*-indole-5-carbaldehyde (1f)



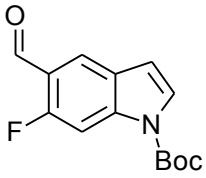
A solution of 80 mg (0.5 mmol) of 6-fluoro-1*H*-indole-5-carbaldehyde (**1d**) in 5 mL DMF was cooled to 0 °C, and 30 mg (0.75 mmol) of a 60% dispersion of NaH in mineral oil were added. The resulting solution was allowed to stir for 20 min at 0 °C, before 100 µL (1.6 mmol) iodomethane were added. The reaction mixture was allowed to reach room temperature and then stirred for further 12 h. Water was added followed by repeated extraction with Et_2O. The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. Purification

by column chromatography gave 52 mg (0.29 mmol, 58 %) of 1-methyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1f**) as an off white solid.

M.p.: 108 – 110 °C

^{1}H-NMR (DMSO) δ 10.21 (s, 1H), 8.09 (d, 1H, J = 6.8 Hz), 7.52-7.46 (m, 2H), 6.67 (dd, 1H, J = 0.9 Hz, J = 3.2 Hz), 3.82 (s, 3H); ^{13}C-NMR (DMSO) δ 188.18 (J = 5.3 Hz), 160.29 (J = 245.8), 140.16 (J = 13.0 Hz), 133.14 (J = 3.1 Hz), 125.09 (J = 0.6 Hz), 123.46 (J = 4.0 Hz), 117.79 (J = 10.8 Hz), 103.38, 97.33 (J = 25.2 Hz, 33.41; ^{19}F-NMR (DMSO) δ -129.49; HRMS: $C_{10}H_8FNO$ $[M+Na]^+$ calcd. 200.0482 found 200.0482; E.A.: calcd. C: 67.79 H: 4.55 N: 7.91 found C: 66.42 H: 4.61 N: 7.61

1-Boc-6-fluoro-1*H*-indole-5-carbaldehyde (1g), according to Koning et al.[159]

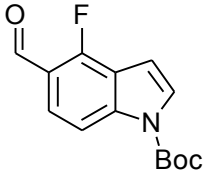


To a solution of 100 mg (0.61 mmol) of 6-fluoro-1*H*-indole-5-carbaldehyde (**1d**) in 7 mL THF were added 7.3 mg (0.06 mmol) DMAP and 152 mg (0.7 mmol) Boc_2O. The resulting solution was stirred for 4 h at room temperature. Water was added followed by repeated extraction with Et_2O. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. Purification by column chromatography gave 136 mg (0.48 mmol, 72 %) of 1-Boc-6-fluoro-1*H*-indole-5-carbaldehyde (**1g**) as a colorless solid.

M.p.: 91 -93 °C

^{1}H-NMR (DMSO) δ 10.38 (s, 1H), 8.05 (d, 1H, J = 6.7 Hz), 7.95 (d, 1H, J = 11.2 Hz), 7.62 (d, 1H, J = 3.6 Hz), 6.63 (d, 1H, J = 3.8 Hz), 1.68 (s, 9H); ^{13}C-NMR (DMSO) δ 187.43 (J = 6.8 Hz), 162.63 (J = 249.8 Hz), 148.97, 139.08 (J = 13.2 Hz), 127.96 (J = 3.1 Hz), 127.01, 121.33 (J = 3.1 Hz), 120.27 (J = 10.1 Hz), 107.81, 102.95 (J = 28.1 Hz), 85.03, 28.10; ^{19}F-NMR (DMSO) δ -126.44; HRMS: $C_{16}H_{12}FNO_3S$ $[M+Na]^+$ calcd. 286.0849 found 286.0849; E.A.: calcd. C: 63.87 H: 5.36 N: 5.32 found C: 63.71 H: 5.38 N: 5.23

1-Boc-4-fluoro-1*H*-indole-5-carbaldehyde (2g), according to Koning et al.[159]

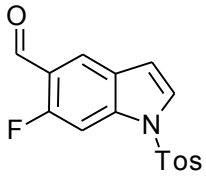


Analogously prepared as the aldehyde **1e**, but starting from 7-fluoro-1*H*-indole-6-carbaldehyde (**3d**) giving the desired product as a colorless solid in 82 % yield.

M.p.: 112 – 114 °C

^{1}H-NMR ($CDCl_3$) δ 10.48 (s, 1H), 8.06 (d, J = 8.7 Hz, 1H), 7.82 (dd, J = 8.7 Hz, J = 6.5 Hz, 1H), 7.67 (d, J = 3.8 Hz, 1H), 6.81 (d, J = 3.8 Hz, 1H), 1.73 (s, 9H); ^{13}C-NMR ($CDCl_3$) δ 186.91 (J = 7.0 Hz), 158.72 (J = 247.38), 149.84, 141.57, 127.28, 123.65 (J = 1.62 Hz), 119.27 (J = 20.7 Hz), 118.20 (J = 5.5 Hz), 111.94 (J = 3.8 Hz), 103.41, 85.24, 28.11; ^{19}F-NMR ($CDCl_3$) δ -129.58; HRMS: $C_{16}H_{12}FNO_3S$ $[M+Na]^+$ calcd. 286.0849 found 286.0850; E.A.: calcd. C: 62.77 H: 5.36 N: 5.32 found C: 63.31 H: 5.22 N: 5.21

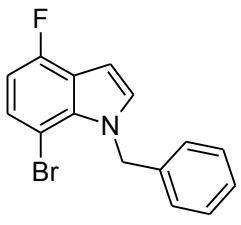
1-Tosyl-6-fluoro-1*H*-indole-5-carbaldehyde (1h), according to Konas et al. [160]



A solution of 150 mg (0.92 mmol) of 6-fluoro-1*H*-indole-5-carbaldehyde (**1d**) in 5 mL DMF was cooled to 0 °C, and 55 mg (1.38 mmol) of a 60% dispersion of NaH in mineral oil were added. The resulting solution was allowed to stir for 20 min at 0 °C, before 263 mg (1.38 mmol) tosyl chloride was added. The reaction mixture was allowed to reach room temperature and stirred for further 12 h. Water was added followed by repeated extraction with Et_2O. The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. Purification by column chromatography gave 239 mg (0.75 mmol, 82 %) of 1-tosyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1h**) as an off white solid.

^{1}H-NMR (DMSO) δ 10.15 (s, 1H), 8.06 (d, 1H, J = 6.7 Hz), 7.94 (d, 2H, J = 9.2 Hz), 7.90 (d, 1H, J = 3.5 Hz), 7.81 (d, 1H, J = 11.4 Hz), 7.37 (d, 1H, J = 8.0 Hz), 6.93 (d, 1H, J = 3.4 Hz), 2.27 (s, 3H); ^{13}C-NMR (DMSO) δ 187.95 (J = 5.4 Hz), 161.43 (J = 251.0 Hz), 146.60, 137.63 (J = 13.0 Hz), 134.06, 130.91, 129.44 (J = 3.5 Hz), 127.42 (J = 4.2 Hz), 127.39, 123.69 (J = 3.6 Hz), 121.04 (J = 10.6 Hz), 110.24, 101.35 (J = 27.3 Hz), 21.47; ^{19}F-NMR (DMSO) δ -124.12; HRMS: $C_{16}H_{12}FNO_3S$ $[M+H]^+$ calcd. 318.0595 found 318.0594; E.A.: calcd. C: 60.56 H: 3.81 N: 4.41 found C: 60.35 H: 3.95 N: 4.46

1-Benzyl-7-bromo-4-fluoro-1*H*-indole (4d)

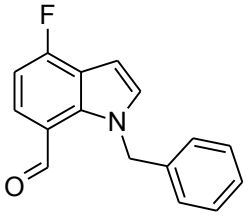


A solution of 612 mg (2.9 mmol) of 7-bromo-4-fluoro-1*H*-indole in dry DMF was cooled to 0 °C, and 173 mg (4.3 mmol) of a 60% dispersion of NaH in mineral oil was added. After 20 min 510 µL (735 mg, 4.3 mmol) benzyl bromide were added and the reaction mixture was allowed to warm up to room temperature and stirred overnight. Water

was added, and the aqueous phase was repeatedly extracted with Et_2O. The combined organic layers were dried over Na_2SO_4, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography yielding 711 mg (2.4 mmol, 81 %) of 1-benzyl-7-bromo-4-fluoro-1*H*-indole (**4d**) as colorless oil.

^{1}H-NMR (DMSO) δ 7.33-7.25 (m, 4H), 7.12-7.03 (m, 3H), 6.71 (dd, 2H, J = 10.5 Hz, J = 9.4 Hz), 5.87 (s, 2H); HRMS: $C_{15}H_{11}BrFN$ $[M+Na]^+$ calcd. 304.0131 found; no corresponding peak could be detected; E.A.: calcd. C: 63.87 H: 5.36 N: 5.32 found C: 63.71 H: 5.38 N: 5.23

1-Benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (4e), according to Boymond et al.[191]



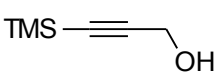
A solution of 420 mg (1.4 mmol) of **4d** in 5 mL THF was cooled to -78 °C, and 560 µL (1.4 mmol) of a 2.5 M solution of *n*-BuLi in hexanes were added. The mixture was stirred for 30 min, before 500 µL DMF were added, followed by warming to room temperature and stirring for 3 h. Water was added, and the aqueous phase was repeatedly extracted with Et_2O. The combined organic fractions were dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography yielding 134 mg (0.5 mmol, 39 %) of 1-benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (**4e**) as a colorless solid.

M.p.: 75 – 77 °C

^{1}H-NMR (DMSO) δ 10.05 (s, 1H), 7.81 (dd, J = 8.4 Hz, J = 5.7 Hz, 1H), 7.71 (d, J = 3.2 Hz, 1H), 7.21-7.10 (m, 6H), 6.98 (t, J = 21.0 Hz, 1H), 6.90 (d, J = 1.3 Hz), 6.82 (d, J = 3.3 Hz), 5.97 (s, 2H); ^{13}C-NMR (DMSO) δ 190.33, 159.89 (J = 154.01 Hz), 138.62, 136.15 (J = 12.9 Hz), 132.14, 132.05 (J = 9.8 Hz), 129.09, 127.73, 126.50, 120.46, 105.39 (J = 19.7 Hz), 98.85, 53.72, 28.54; ^{19}F-NMR (DMSO) δ -111.47; HRMS: $C_{15}H_{11}BrFN$ $[M+Na]^+$ calcd. 304.0131 found; no corresponding peak could be detected; E.A.: calcd. C: 63.87 H: 5.36 N: 5.32 found C: 63.71 H: 5.38 N: 5.23

4.3 Synthesis of the precursor for L-6-fluorotryptophan by build-up synthesis

3-(Trimethylsilyl)prop-2-yn-1-ol (17), as described by Ma et al.[179]

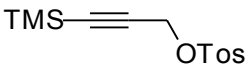


To a solution of propargyl alcohol (1.0 mL, 17.2 mmol) in 45 mL THF were added 21.0 mL of a 2.5 M solution of *n*-BuLi in hexanes at -78 °C.

The solution was stirred for 30 min at –78 °C before 7.7 mL (60 mmol) TMS-Cl was added. The reaction mixture was warmed to room temperature and stirred for one more hour. 25 mL water and 25 mL 10 % hydrochloric acid were subsequently added and the mixture was repeatedly extracted with Et_2O. The organic layers were dried over Na_2SO_4 and the solvents were removed under reduced pressure. Purification by flash chromatography gave the desired product **17** in 97 % yield as a colorless oil. Rf = 0.25 (PE/EA = 9:1)

^{1}H-NMR ($CDCl_3$) δ 4.30 (s, 2H), 1.89 (broad s, 1H), 0.20 (s, 9H)

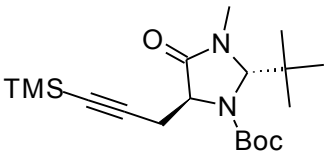
3-(Trimethylsilyl)prop-2-yn-1-tosylate (18), according to by Ma et al.[179]



A solution of 500 mg (3.9 mmol) of the alcohol **17** in Et_2O was cooled to –50 °C before tosyl chloride (900 mg, 4.7 mmol) was added. The solution was stirred for 15 min followed by the addition of powdered KOH (700 mg, 12.4 mmol). The reaction mixture was slowly warmed to 0 °C and stirred for about 1.5 h at this temperature. 30 mL ice water were added and the aqueous phase was extracted with Et_2O. The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification via flash chromatography gave the desired tosylate **18** in 87 % (960 mg, 3.4 mmol) yield as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ7.86 (d, 2H, J = 8.3 Hz), 7.33 (d, 2H, J = 12.0 Hz), 4.75 (s, 2H), 2.49 (s, 3H), 0.12 (s, 9H)

(S,S)2-Tert-butyl-3-methyl-4-oxo-5-[3-(trimethylsilyl)prop-2-yn-1-yl]imidazolidine-1-carboxylate (15)

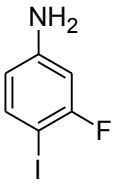


At -78 °C *n*-BuLi in hexane (0.73 mL, 1.8 mmol) was added to a solution of diisopropylamine (256 µL, 1.8 mmol) in 1.5 mL THF. The resulting solution was stirred for 30 min, before a solution of Boc-BMI (380 mg, 1.5 mmol) in 3 mL THF was added. After another 30 min of stirring at -78 °C a solution of **18** in 1.5 mL THF was added, and the reaction mixture was allowed to reach room temperature. After stirring for 3 h, the reaction was quenched with water and the aqueous phase was repeatedly extracted with Et_2O. The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. Purification by flash chromatography (PE/EA = 4:1, R_f = 0.29) gave the desired product **15** in 82 % (429 mg, 1.2 mmol) yield as a colorless solid.

M.p.: 133- 135 °C

^{1}H-NMR ($CDCl_3$) δ 5.09 (s, 1H), 4.02 (s, 1H), 3.36 (d, 1H, J = 14.2 Hz) 3.10 (s, 3H), 2.88 (d, 1H, J = 16.6 Hz), 1.55 (s, 9H), 1.00 (s, 9H), 0.12 (s, 9H)

3-Fluoro-4-iodoaniline (20), according to Emmanuvel et al.[180]

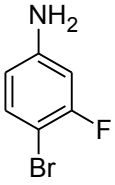


KI (1.46 g, 8.8 mmol) was added to a solution of 2-fluoroaniline (1.0 g, 8.8 mmol), $NaIO_4$ (1.88 g, 8.8 mmol) and NaCl (1.0 g, 17.2 mmol) in a mixture of acetic acid and water and the reaction mixture was stirred for 3 h at room temperature. 30 mL ice water was added, and the aqueous phase was extracted several times with DCM. The combined organic extracts were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Purification by flash chromatography gave the desired aniline **20** in 82 % (1.7 g, 7.2 mmol) yield as a yellow solid.

M.p.: 65 – 67 °C (Lit. 67 – 68.5 °C[203])

^{1}H-NMR ($CDCl_3$) δ 7.45 (dd, 1H, J = 8.5 Hz, J = 7.2 Hz), 6.46 (dd, 1H, J = 9.9 Hz, J = 2.6 Hz), 6.31 (dd, 1H, J = 8.3 Hz, J = 2.5 Hz), 3.85 (broad s, 2H)

3-Fluoro-4-bromoaniline (22), according to Majetich et al.[182]

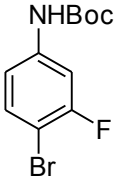


To a solution of 3-fluoroaniline (4.0 g, 36 mmol) in 15 mL DMSO was added 5 mL aqueous HBr (48 %) at about 10 °C. The reaction mixture was stirred for 6 h at room temperature before water was added. Afterwards NaOH was added until the solution was basic. The aqueous phase was repeatedly extracted with Et_2O and the combined organic extracts were dried over Na_2SO_4. The solvent was evaporated under reduced pressure. Flash chromatography (PE/EA = 4:1, R_f = 0.26) of the crude mixture provided 3-fluoro-4-bromoaniline (**22**) as a yellowish solid in 82 % (5.5 g, 29 mmol) yield.

M.p.: 58 - 61 °C (Lit. 63 65 °C[204])

^{1}H-NMR ($CDCl_3$) δ 7.28 (dd, 1H, J = 8.3 Hz, J = 4.4 Hz), 6.49 (dd, 1H, J = 10.4 Hz, J = 2.6 Hz), 6.39 (dd, 1H, J = 8.6 Hz, J = 2.6 Hz), 3.75 (broad s, 2H

1-Boc-3-fluoro-4-bromoaniline (23) according to Peterson et al.[205]

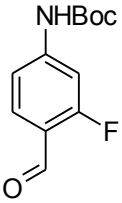


To a solution of 3-fluoro-4-bromo-aninline (**22**) (743 mg, 3.9 mmol) in THF/H_2O (18 mL, 50:50) were added K_2CO_3 (1.0 g, 7.8 mmol) and Boc_2O (850 mg, 3.9 mmol) subsequently. The mixture was stirred overnight at room temperature before it was extracted with Et_2O for several times. The combined organic extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude mixture was purified by flash chromatography giving the desired product in 64 % (725 mg, 2.5 mmol) yield as a yellow solid.

M.p.: 64 – 68 °C

^{1}H-NMR ($CDCl_3$) δ 7.49-7.38 (m, 2H), 6.94 (ddd, J = 8.7 Hz, J = 2.5 Hz, J = 1.0 Hz, 1H), 6.70 (broad s, 1H), 1.55 (s, 9H); ^{13}C-NMR ($CDCl_3$) 161.66, 149.54 (J = 274.6 Hz), 139.35 (J = 10.1 Hz), 133.21 (J = 1.6 Hz), 114.92 (J = 3.3 Hz), 106.89 (J = 27.4 Hz), 101.55 (J = 21.1 Hz), 81.34, 28.30; ^{19}F-NMR ($CDCl_3$) -105.68

1-Boc-3-fluoro-4-formylaniline (25)

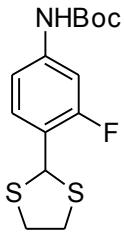


Method A: (according to Boymond et al.[191]) 0.1 mL dioxane was added to a solution of *i*-PrMgCl.LiCl in THF (1.7 mL, 1.7 mmol) at 0 °C. The solution was stirred for 5 min before the bromide **23** (384 mg, 1.3 mmol) in 1 mL THF was added. The reaction mixture was stirred at 0 °C for 1 h. Then 200 µL DMF (2.6 mmol) were added, the solution was warmed to room temperature and stirred for further 3 h at this temperature. A saturated aqueous solution of NH_4Cl was added followed by extraction with Et_2O. The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. Flash chromatography of the crude mixture (PE/EA = 95:5, R_f = 0.14) provided the desired product in 97 % yield as a colorless solid.

Method B: (according to Qin et al.[183]) To a solution of 4-bromo-2-fluorobenzaldeyde (**24**) (500 mg, 2.5 mmol) in dry dioxane were added $Pd(OAc)_2$ (17 mg, 0.075 mmol), XPhos (110 mg, 0.225 mmol), *t*-butylcarbamate (350 mg, 3 mmol) and Cs_2CO_3 (1.1 g, 3.5 mmol). The reaction mixture was heated to 100 °C for 4 h, cooled to room temperature and extracted repeatedly with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. Flash chromatography of the crude mixture (PE/EA = 95:5, R_f = 0.14) provided the desired product **25** in 95 % (560 mg, 2.3 mmol) yield as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ 10.25 (s, 1H), 7.81 (t, 1H, J = 8.5 Hz), 7.59 (dd, 1H, J = 13.0 Hz, J = 2.0 Hz), 7.06 (dd, 1H, J = 8.5 Hz, J = 2.0 Hz), 1.55 (s, 9H); ^{13}C-NMR ($CDCl_3$) δ 186.15 (J = 6.0 Hz), 165.85 (J = 255.3 Hz), 151.79, 146.12 (J = 12.6 Hz), 129.51 (J = 3.6 Hz), 118.93 (J = 8.3 Hz), 113.73 (J = 2.9 Hz), 105.04 (J = 26.4 Hz), 82,01, 28,20; ^{19}F-NMR ($CDCl_3$) δ -119.31

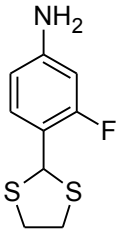
1-Boc-4-(1,3-dithiolan-2-yl)-3-fluoroaniline (26), according to Firouzabadi et al. [186]



To a solution of the benzaldehyde **25** (370 mg, 1.54 mmol) and elemental iodine (20 mg, 0.16 mmol) in DCM 155 µL (1.84 mmol) 1,2-ethanedithiol were added. The mixture was stirred at room temperature for 1 h before water was added. The aqueous phase was repeatedly extracted with DCM. The combined organic extracts were dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. Purification of the crude mixture by flash chromatography provided the desired product as a colorless solid in 90 % (437 mg, 1.38 mmol) yield as a slightly yellow solid.

^{1}H-NMR ($CDCl_3$) δ 7.63 (t, 1H, J = 8.5 Hz), 7.34 (dd, 1H, J = 12.4 Hz, J = 2.1 Hz), 6.97 (dd, 1H, J = 8.5 Hz, J = 2.1 Hz), 6.60 (s, 1H), 5.94 (s, 1H), 3.52-3.35 (m, 4H), 1.55 (s, 9H)

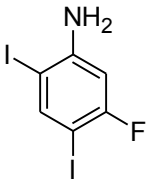
4-(1,3-Dithiolan-2-yl)-3-fluoroaniline (27)



5 mL TFA were added to a solution of the dithiolan **26** (390 mg, 1.23 mmol) in 5 mL DCM. The reaction mixture was stirred for 1 h at room before water was added. Na_2CO_3 was added until the solution was basic followed by repeated extraction with DCM. The combined organic extracts were dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography (PE/EA = 4:1, R_f = 0.23) giving the desired product in 77 % (206 mg, 0.95 mmol) yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ 7.51 (t, 1H, J = 8.5 Hz), 6.45 (dd, 1H, J = 8.4 Hz, J = 2.3 Hz), 6.35 (dd, 1H, J = 12.0 Hz, J = 2.3 Hz), 5.94 (s, 1H), 3.85 (s, 2H), 3.55-3.28 (m, 4H)

3-Fluoro-4,6-diodoaniline (14), according to Emmanuvel et al.[180]



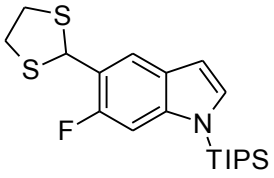
KI (2.9 g, 17.6 mmol) was added to a solution of 2-fluoroaniline (1.0 g, 8.8 mmol), $NaIO_4$ (1.88 g, 8.8 mmol) and NaCl (1.0 g, 17.2 mmol) in a mixture of

acetic acid and water and the reaction mixture was stirred for 6 h at room temperature. 30 mL ice water were added and the aqueous phase was extracted several times with DCM. The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. Purification by flash chromatography gave the desired aniline **29** in 88 % (2.8 g, 7.7 mmol) yield as a brown solid.

^{1}H-NMR ($CDCl_3$) δ 7.93 (d, 1H, J = 6.8 Hz), 6.54 (d, 1H, J = 9.6 Hz), 4.29 (broad s, 2H); FT-MS (ESI) $C_6H_4FI_2N$ m/z $[M+H]^+$ calcd.: 363.849 found: 363.80

4.4 Synthesis of the precursor of L-6-fluorotryptophan by linear synthesis

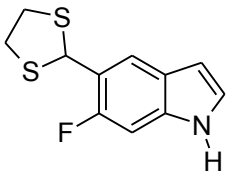
5-(1,3-Dithiolan-2-yl)-6-fluoro-1-triisopropylsilyl-1*H*-indole (31)
according to Firouzabadi et al. [186]



To a solution of the indolecarbaldehyde **1c** (2.5g, 7.9 mmol) and elemental iodine (100 mg, 0.79 mmol) in DCM were added 120 µL (9.5 mmol) 1,2-ethanedithiol. The reaction mixture was stirred for 1 h at room temperature and quenched with 40 mL 0.1 M $Na_2S_2O_3$ and 40 mL 0.1 M NaOH. The aqueous phase was extracted repeatedly with DCM, the organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography giving the desired product as a colorless solid in 40 % (1.23 g, 3.1 mmol) yield.

^{1}H-NMR ($CDCl_3$) δ 7.98 (d, 1H, J = 7.8 Hz), 7.26-7.17 (m, 2H), 6.63 (dd, 1H, J = 3.3 Hz, J = 0.9 Hz), 6.11 (s, 1H), 3.61-3.19 (m, 4H), 1.71 (hept, 3H, J = 7.7 Hz), 1.18 (d, 18H, J = 7.4 Hz)

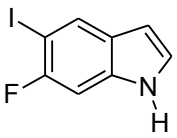
5-(1,3-Dithiolan-2-yl)-6-fluoro-1*H*-indole (32), according to Schlosser et al.[69]



1 mL of a 1.0 M solution of TBAF in THF was added to a solution of the dithiolan **31** (1.2 g, 3 mmol) in THF. The mixture was stirred at room temperature for about 10 min before the solvent was evaporated. The crude product was purified by flash chromatography giving the desired indole **32** as a colorless solid in 92 % (655 mg, 2.74 mmol) yield.

^{1}H-NMR ($CDCl_3$) δ 8.11 (broad s, 1H), 7.98 (d, 1H, J = 7.4 Hz), 7.16 (t, 1H, J = 3.0 Hz), 7.03 (d, 1H, J = 10.9 Hz), 6.53-6.50 (m, 1H), 6.07 (s, 1H), 3.57-3.30 (m, 4H)

6-Fluoro-5-iodo-1*H*-indole (35), as described by Schlosser et al.[69]

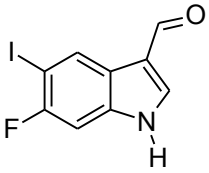


sec-BuLi in hexane (20 ml, 28 mmol, 1.4 M) was added to solution of 8.0 g (27.5 mmol) 6-fluoro-1-triisopropyl-1*H*-indole (**1b**) and 5.8 ml N,N,N',N'',N''-pentamethyldiethylenetriamine (4.9 g, 27.5 mmol) in 40 mL THF at –78 °C. The solution was stirred at this temperature for 6 h before 7.8 g (27.5 mmol) of 1,2-diiodoethane were added. The reaction mixture was slowly warmed up to room temperature and stirred overnight. Water was added, and the aqueous phase was repeatedly extracted with Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product **34**, a brownish oil, was dissolved in 30 ml THF and 7.1 g (27.1 mmol) TBAFxH_2O were added. The mixture was stirred for 30 min at RT before water was added. The aqueous phase was extracted several times with Et_2O. The organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated *in vacuo*. The crude residue was purified by flash chromatography (PE/EA = 4:1; R_f = 0.45) giving 4.7 g (17.9 mmol, 65 %) of the desired product as a light green solid.

M.p.: 32 – 36 °C (Lit. 37 – 39 °C[69])

^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.21 (br s, 1H), 8.02 (d, 1H, J = 6.1 Hz), 7.30-7.16 (m, 2H), 6.53-6.50 (m, 1H)

6-Fluoro-5-iodo-1*H*-indole-3-carbaldehyde (36), according to Konas et al.[160]



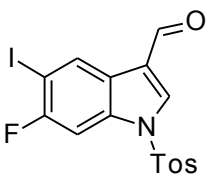
At 0 °C 5.7 mL $POCl_3$ (35.5 mmol) were added to 16.0 mL DMF. The mixture was stirred at this temperature for 15 min before 4.1 g (15.6 mmol) of 6-fluoro-5-iodo-1*H*-indole (**35**) in 37 mL DMF were added. The solution was warmed up to RT and stirred until it became an opaque paste (approximately 1 h). 10 % $NaOH_{aq}$ was added until the pH of the solution was greater than 10. The suspension was heated to reflux for 10 min before it was cooled to 0 °C. The precipitate was filtered and washed with water and Et_2O. After drying under reduced pressure at 50 °C, the desired product was obtained as an off white solid (64 %, 2.9 g, 10.0 mmol).

M.p.: decomposition at 289 – 291 °C

^{1}H-NMR (400 MHz, DMSO) δ 12.28 (s, 1H), 9.90 (s, 1H), 8.47 (d, 1H, J = 6.3 Hz), 8.32 (s, 1H), 7.44 (d, 1H, J = 8.7 Hz); ^{13}C-NMR (400 MHz, DMSO) δ 185.44, 157.77 (J = 234.5 Hz), 139.89 (J = 1.9 Hz), 137.40 (J = 11.4 Hz), 130.46 (J = 3.2 Hz), 123.31 (J = 1.0 Hz), 117.22,

99.88 (J = 28.9 Hz), 75.72 (J = 28.0 Hz); ^{19}F-NMR (400 MHz, DMSO) δ -100.44; HRMS: $C_8H_7FINO_2$ $[M+H]^+$ calcd. 289.9473 found 381.2975; E.A.: calcd. C: 37.40 H: 1.74 N: 4.85 found C: 37.54 H: 1.88 N: 4.86

6-Fluoro-5-iodo-1-tosyl-1*H*-indole-3-carbaldehyde (37a), according to Konas et al.[160]

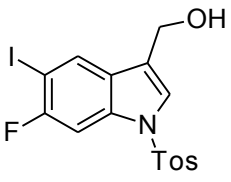


A solution of 3.8 g (13.3 mmol) of 4-fluoro-5-iodo-1*H*-indole-3-carbaldehyde (**36**) in 40 mL THF was cooled to 0 °C and 480 mg (20 mmol) of a 60 % dispersion of NaH in mineral oil was added. The resulting solution was stirred for 20 min at 0 °C before 3.8 g (20 mmol) Tos-Cl were added. The reaction mixture was warmed to RT and stirred for another 2 h. Water was added and the aqueous phase was repeatedly extracted with DCM. The combined organic extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo*. Purification by flash chromatography (PE/EA = 4:1 R_f = 0.38) gave the desired product as a colorless solid (88 %, 5.2 g, 11.7 mmol).

M.p.: 191-192 °C

^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H), 8.65 (d, 1H, J = 6.2 Hz), 8.19 (s, 1H), 7.83 (d, 2H, J = 8.1 Hz), 7.69 (d, 1H, J = 8.3 Hz), 7.32 (d, 2H, J = 8.1 Hz), 2.39 (s, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 184.70, 159.51 (J = 242.0 Hz), 146.63, 136.51 (J = 3.2), 135.25 (J = 11.2 Hz), 133.71, 132.60 (J = 2.8 Hz), 130.47, 127.13, 124.16 (J = 2.0 Hz), 120.86 (J = 0.9 Hz), 100.71 (J = 31.3 Hz), 78.44 (J = 27.3 Hz), 21.63 ; ^{19}F-NMR (400 MHz, $CDCl_3$) δ -93.31; HRMS: $C_{16}H_{11}FINO_3S$ $[M+H]^+$ calcd. 443.9561 found 443.9561; E.A.: calcd. C: 43.36 H: 2.50 N: 3.16 found C: 43.05 H: 2.59 N: 3,11

[6-Fluoro-5-iodo-1-tosyl-1*H*-indol-3-yl]methanol (38a), according to Konas et al.[160]

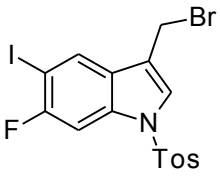


541 mg (14.3 mmol) $NaBH_4$ were added to a solution of 4.2 g of **37** (9.5 mmol) in 150 mL THF/EtOH (2:1) at 0 °C. The resulting solution was warmed to RT and stirred for 30 min. Saturated NH_4Cl_{aq} was added and the mixture was extracted with DCM. The combined organic extracts were washed with brine, dried over Na_2SO_4 and filtered. Evaporation of the solvent gave the desired product as a colorless solid (97 %, 4.1 g, 9.2 mmol).

M.p.: 171-172 °C

^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.13 (d, 1H, J = 6.3 Hz), 7.92 (d, 2H, J = 8.3 Hz), 7.81 (d (1H, J = 8.9 Hz), 7.70 (s, 1H), 7.41 (d, 2H, J = 8.5 Hz), 5.23 (t, 1H, J = 5.6 Hz), 4.58 (d, 2H, J = 5.4 Hz), 2.34 (s, 3H); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 158.77 (J = 136.7 Hz), 146.32, 135.29, 135.06. 134.26, 130.80, 128.61, 127.33, 125.00 (J = 3.8 Hz), 123.53 (J = 1.3 Hz), 101.82 (J = 31.3 Hz), 77.54 (J = 28.1 Hz), 55.23, 21.53; ^{19}F-NMR (200 MHz, $CDCl_3$) δ -97.75; HRMS: $C_{16}H_{13}FINO_3S$ $[M+H]^+$ calcd. 445.9718 found 427.9612 (ohne OH); E.A.: calcd. C: 43.16 H: 2.94 N: 3.15 found C: 43.32 H: 3.01 N: 3.13

[3-(Bromomethyl)-6-fluoro-5-iodo-1-tosyl]-1*H*-indole (39a), according to Appel et al.[189]

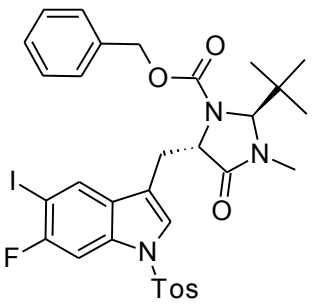


A solution of the alcohol **38** (2.0 g, 4.5 mmol) was cooled to 0 °C before PPh_3 (1.3 g, 5.0 mmol) and CBr_4 (1.7 g, 5.0 mmol) were added subsequently. The mixture was stirred at 0 °C and monitored by TLC (PE/EA = 4:1, R_f = 0.72). After approximately 30 min the reaction was finished and the solvent was partially removed *in vacuo* at room temperature. The oily residue was applied to a silica column and eluted with a mixture of PE/EA (4:1). Removal of the solvents under reduced pressure gave the desired product as a colorless solid (64 %, 1.4 g, 2.8mmol).

M.p.: decomposition at 184 – 186 °C

^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.03 (d, 1H, J = 5.9 Hz), 7.82-7.74 (m, 2H), 7.64 (s, 1H), 7.32 (d, 2H, J = 7.3 Hz), 4.53 (s, 2H), 2.42 (s, 3H); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 519.30 (J = 240.5 Hz), 145.87, 135.53 (J = 11.4 Hz), 134.55, 130.28, 129.95, 129.89, 127.33, 127.29, 126.95, 125.81 (J = 3.9 Hz), 117.80 (J = 1.4 Hz), 101.34 (J = 31.2 Hz), 22.77 (J = 8.7 Hz); ^{19}F-NMR (200 MHz, $CDCl_3$) δ -95.54; HRMS: $C_{16}H_{12}BrFINO_2S$ $[M+Na]^+$ calcd. 529,8693 found 529.8696, 531.8676 ($C_{16}H_{12}{}^{81}BrFINO_2S$); E.A.: calcd. C: 37.82 H: 2.38 N: 2.76 found C: 37.79 H: 2.49 N: 2.51

Benzyl (2S,5S)-2-*tert*-butyl-5-[(6-fluoro-5-iodo-1-tosyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimidazolidine-1-carboxylate (40)



n-BuLi in hexane (1.24 mL, 3.1 mmol) was added to a solution of diisopropylamine (435 µL, 3.1 mmol) in 3 mL THF at -78 °C. The resulting solution was stirred for 30 min before a solution of Z-BMI (772 mg, 2.7 mmol) in 3 ml THF was added. After another 30 min

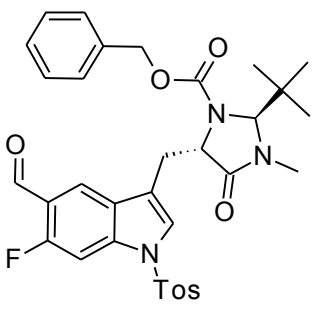
of stirring at -78 °C a solution of the bromide **39** in 4.5 mL THF was added. The reaction was slowly warmed to RT and stirred overnight before it was quenched with saturated NH_4Cl_{aq}. Water was added and the aqueous phase repeatedly extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure. Purification of the crude product by flash chromatography (PE/EA = 7:3; R_f = 0.4) gave the desired product as a colorless solid (74 %, 1.4 g, 2.0 mmol).

M.p.: 75 – 78 °C

^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.43 (s, 1H),

^{13}C-NMR (200 MHz, $CDCl_3$) δ 171.52, 145.28, 134.90, 134,86, 134,68, 130.04, 129.42, 129.39, 126.91, 115.65, 100.96, 100.33, 81.61, 81.19, 31.83, 28.21, 26.57, 21.59; ^{19}F-NMR (200 MHz, $CDCl_3$) δ -95.54; HRMS: $C_{16}H_{12}BrFINO_2S$ $[M+Na]^+$ calcd. 529.8693 found 529.8696, 531.8676 ($C_{16}H_{12}{}^{81}BrFINO_2S$); E.A.: calcd. C: 37.82 H: 2.38 N: 2.76 found C: 37.79 H: 2.49 N: 2.51

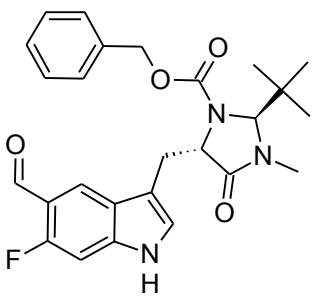
Benzyl (2S,5S)-2-*tert*-butyl-5-[(6-fluoro-5-formyl-1-tosyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimidazolidine-1-carboxylate (41), according to Boymond et al.[191]



1.7 mL of a 2.9 M solution of *i*-PrMgBr in THF were added to a solution of the iodide **40** (1.8 g, 2.5 mmol) in 5 mL THF at 0 °C. The resulting solution was stirred for 1 h at 0 °C before 770 µL DMF (10 mmol) were added. The reaction was warmed up to room temperature and stirred for another 3 h. Saturated NH_4Cl_{aq} was added, and the aqueous phase was extracted several times with Et_2O. The combined organic extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure. Flash chromatography (PE/EA = 7:3, R_f = 0.27) of the crude mixture gave the desired product as a colorless solid (85 %, 1.3 g, 2.1 mmol).

^{1}H-NMR (200 MHz, $CDCl_3$) δ 10.31 (s, 1H), 7.76-7.67 (m, 3H), 7.41-7.20 (m, 8H), 6.95 (ddd, 1H, J = 9.0 Hz, J = 9.0 Hz, J = 2.5 Hz), 5.11 (q, 2H, J = 11.8 Hz), 4.60 (s, 1H), 4.38 (s, 1H), 3.94 (s, 1H), 3.29 (d, 1H, J = 14.3 Hz), 3.08 (s, 1H), 2.64 (s, 3H), 2.36 (s, 3H), 0.91 (s, 9H); ^{19}F-NMR (200 MHz, $CDCl_3$) δ -124.90; HRMS: $C_{33}H_{34}FN_3O_6S$ $[M+H]^+$ calculated 620.2225 found 620.2227; E.A.: calculated C: 63.96 H: 5.53 N: 6.78 found C: 64.42 H: 4.96 N: 7.42

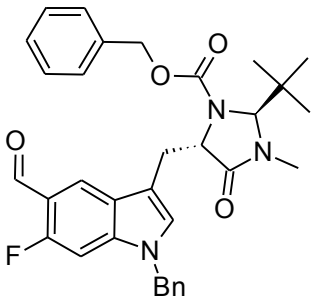
Benzyl (2S,5S)-2-*tert*-butyl-5-[(6-fluoro-5-formyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxo-imidazol-idine-1-carboxylate (42), according to Yasuhara et al.[170]



3.5 mL of 1.0 M solution of TBAF in THF were added to a solution of 432 mg (0.7 mmol) **41** in 10 mL THF. The resulting mixture was heated to reflux for 3 h, cooled to room temperature, and the solvent was evaporated *in vacuo*. Flash chromatography of the crude mixture gave the desired product as a colorless solid in 91 % (297 mg, 0.64 mmol) yield.

^{1}H-NMR ($CDCl_3$) δ 8.04-7.65 (m, 3H), 7.44-7.15 (m, 9H), 5.10 (d, J = 3.6 Hz, 2H), 4.62 (s, 1H), 4.38 (s, 1H), 3.88 (s, 1H), 3.24 (d, J = 14.7 Hz, 1H), 2.77 (s, 3H), 2.38 (s, 3H), 0.92 (s, 9H); ^{19}F-NMR ($CDCl_3$) δ -95.67; HRMS $C_{26}H_{28}FN_3O_4$ $[M+H]^+$ calculated 466.2137 found 466.2141

Benzyl (2S,5S)-2-*tert*-butyl-5-[(1-benzyl-4-fluoro-5-formyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimidazolidine-1-carboxylate (43)



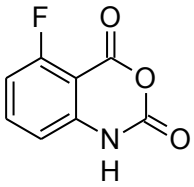
A dispersion of NaH in mineral oil (16 mg, 0.4 mmol) was added to a solution of **42** (200 mg, 0.38 mmol) in 5 mL DMF at 0 °C. The reaction mixture was stirred at 0 °C before 70 µL benzyl bromide were added, and the mixture was warmed to room temperature and stirred for 3 h. Water was added, and the aqueous phase was repeatedly extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude mixture was purified via flash chromatography giving the desired precursor **43** as a colorless solid in 62 % yield (146 mg, 0.24 mmol)

M.p.: 152 – 154 °C

^{1}H-NMR ($CDCl_3$) δ 10.30 (s, 1H), 8.09 (d, J = 6.2 Hz), 7.37-7.12 (m, 10H), 6.98-6.70 (m, 3H), 5.18 (s, 2H), 4.62 (s, 2H), 4.48 (s, 1H), 4.41 (s, 1H), 3.92 (broad s, 1H), 3.32 (d, J = 18.8 Hz), 2.65 (s, 3H), 0.90 (s, 9H); ^{19}F-NMR (200 MHz, $CDCl_3$) δ -122.88; HRMS $C_{33}H_{34}FN_3O_4$ $[M+H]^+$ calculated 556.2606 found 556.2608; E.A.: calculated: C: 71.33 H: 6.17 N: 7.56 found: C: 71.86 H: 5.88 N: 7.08

4.5 Synthesis of the precursor of L-4-fluorotryptophan by build-up synthesis

5-Fluoro-*2H*-3,1-benzoxazine-2,4(1*H*)-dione (47), according to Tedesco et al.[194]

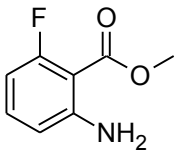


10.0 g (64.5 mmol) 2-amino-6-fluorobenzoic acid (**46**) were dissolved in 140 mL THF and 9.5 g (32.6 mmol) triphosgen were added. The mixture was stirred at 50 °C overnight, allowed to cool to room temperature and filtered. The resulting solid was washed with Et_2O for several times and dried *in vacuo* at 40 °C to yield 10.47 g (58 mmol, 90%) 5-fluoro-*2H*-3,1-benzooxazine-2,4(1*H*)-dione (**47**) as an off white solid.

M.p.: decomposition at 251 – 259 °C

^{1}H-NMR (200 MHz, DMSO) δ 11.89 (s, 1H), 7.74 (td, 1H, J = 8.3 Hz,J = 8.3 Hz, J = 5.7 Hz), 7.10-6.95 (m, 2H); ^{13}C-NMR (200 MHz, DMSO) δ 164.69, 159.49, 155.95 (J = 4.8 Hz), 147.33, 143.53 (J = 2.6 Hz), 138.46 (J = 11.1 Hz), 111.73 (J = 3.9 Hz), 110. 60 (J = 20.0 Hz); ^{19}F-NMR (200 MHz, DMSO) δ -110.25; Ms (+c ESI): $C_8H_4FNO_3$ $[M + H^+]^+$calculated 181.12 found 181.97, 213.90 $[M + Na^+]^+$

Methyl 6-amino-2-fluoro-benzoate (48), according to Levesque et al. [193]

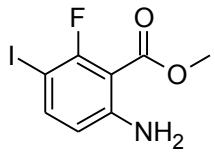


580 mg (4.8 mmol) DMAP were added to a solution of 8.7 g (48 mmol) **47** in 300 ml dry MeOH. The resulting mixture was heated to reflux for 24 h, allowed to cool to room temperature, and the solvent was removed *in vacuo*. The residue was taken up in Et_2O, the organic phase washed with water, dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. The residue was purified by column chromatography (PE/EA = 4:1, R_f = 0.74) giving 7.8 g (46.1 mmol, 97 %) of the desired product as a colorless solid.

M.p.: decomposition at 161 – 166 °C

^{1}H-NMR (200 MHz, DMSO) δ 7.20 (td, 1H, J = 8.2 Hz, J = 8.2 Hz, J = 6.2 Hz), 6,63-6,58 (m, 3H), 6.32 (ddd, 1H, J = 11.7 Hz, J = 8.0 Hz, J = 1.1 Hz), 3.82 (s, 3H); ^{13}C-NMR (200 MHz, DMSO) δ 166.74 (J = 3.3 Hz), 165.37, 160.35, 152.67 (J = 4.8 Hz), 134.11 (J = 12.1 Hz), 112.55 (J = 2.9 Hz), 102.16 (J = 23.3 Hz), 52.17 (J = 0.6 Hz); ^{19}F-NMR (200 MHz, DMSO) δ -108.21; Ms (+c ESI): m/z = 169.99 $[M + H^+]^+$

Methyl 6-amino-2-fluoro-3-iodobenzoate (49), according to DeVita et al. [195]

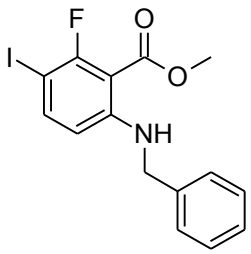


12.7 g (41 mmol) Ag_2SO_4 and 5.2 g (41 mmol) I_2 were consecutively added to a solution of 6.9 g (41 mmol) of the methyl ester **48** in 100 ml EtOH. The reaction mixture was stirred for 3 h at room temperature, the solid was filtered off and the filtrate concentrated *in vacuo*. The residue was taken up in EtOAc, and the organic phase was washed with saturated $NaHCO_3$, brine and water, dried over Na_2SO_4, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography to yield 9.8 g (32.9 mmol, 80 %) of the desired iodo benzoate **49** as a colorless solid.

M.p: 133 – 134 °C

^{1}H-NMR (200 MHz, DMSO) δ 7.53 (dd, 1H, J = 8.9 Hz, J = 7.0 Hz), 6.73 (s, 2H), 6.38 (dd, 1H, J = 8.2 Hz, J = 1.0 Hz), 3.83 (s, 3H); ^{13}C-NMR (200 MHz, DMSO) δ 165.90 (J = 4.0 Hz), 160.95 (J = 248.1 Hz), 152.35 (J = 4.6 Hz), 141.73 (J = 5.1 Hz), 114.79 (J = 3.2 Hz), 101.56 (J = 17.0 Hz), 63.24 (J = 28.4 Hz), 52.33; ^{19}F-NMR (200 MHz, DMSO) δ -87.93; HRMS: $C_8H_7FINO_2$ $[M+H]^+$ calculated 295,9578 found 295,9579; E.A.: calculated C: 32,57 H: 2,39 N: 4,75 found C: 32,68 H: 2,44 N: 4,68

Methyl 6-(benzylamino)-2-fluor-3-iodbenzoate (50), according to Levesque et al.[193]



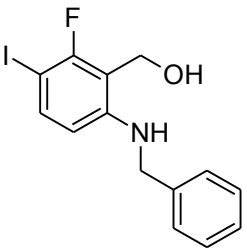
3.0 g (22 mmol) $ZnCl_2$ and 2.8 g (26.2mmol) benzaldehyde were added to a solution of 3.86 g (13.1 mmol) **49** in 30 ml MeOH. The reaction mixture was stirred for 20 min at room temperature before 1.4 g (22 mmol) $NaBH_3CN$ were added. After stirring overnight at room temperature and refluxing for 2 h, the reaction was quenched with 1 M HCl and extracted with DCM. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography gave the desired product as an off white solid (3.9 g, 10.2 mmol, 78 %).

M.p: 106-107 °C

^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.54 (dd, J = 9.0 Hz, J = 6.7 Hz, 1H), 7.40-7.31 (m, 5H), 6.32 (dd, J = 9.1 Hz, J = 1.0 Hz), 4.46 (s, 2H), 3.95 (s, 3H)); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 167.35, 165.79 (J = 146.2 Hz), 151.76 (J = 4.1 Hz), 142.68 (J = 5.1 Hz), 137.85, 128.84, 127.49, 127.03, 109.75 (J = 3.2 Hz), 101.85 (J = 16.0 Hz), 63.88 (J = 29.1 Hz), 52.16, 47.63;

^{19}F-NMR (200 MHz, $CDCl_3$) δ -83.19; HRMS: $C_{15}H_{13}FINO_2$ $[M+H]^+$ calculated 386.0048 found 386.0049; E.A. calculated C: 46.77 H: 3.40 N: 3.64 found C: 45.94 H: 3.35 N: 4.08

[6-(Benzylamino)-2-fluoro-3-iodophenyl]methanol (51a)

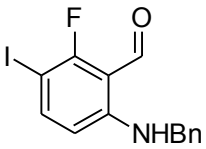


DIBAL (10.4 mL, 10.4 mmol) was added to a solution of the aminobenzoate **50** (2.0 g, 5.2 mmol) in 20 mL THF at 0 °C. The reaction mixture was stirred overnight and quenched with water. Et_2O was added, the precipitate was filtered off and washed with DCM. The organic phase was separated, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Flash chromatography (PE/EA = 9:1; R_f = 0.28) of the crude mixture provided the benzyl alcohol **51a** as a colorless solid in 60 % (1.1 g, 3.1 mmol) yield.

M.p. 83-84 °C

^{1}H-NMR (200 MHz, DMSO) δ 7.41-7.20 (m, 5H), 6.29 (t, J = 5.7 Hz, 1H), 6.20 (d, J = 8.7 Hz, 1H), 5.24 (t, J = 5.5 Hz, 1H), 4.60 (q, J = 1.9 Hz, 2H), 4.40 (d, J = 6.0 Hz, 2H); ^{13}C-NMR (200 MHz, DMSO) δ 159.20 (J = 236.7 Hz), 149.57 (J = 6.4 Hz), 139.95, 137.91 (J = 4.4 Hz), 128.86, 127.44 (J = 5.1 Hz), 127.24, 113.68 (J = 19.3 Hz), 109.55 (J = 2.5 Hz), 64.10 (J = 28.2 Hz), 53.80 (J = 6.2 Hz), 46.77; ^{19}F-NMR (200 MHz, DMSO) δ -101.27; HRMS: $C_{14}H_{13}FINO$ $[M+H]^+$ calculated 358.0099 found 358.0099; E.A. calculated: C: 47.08 H: 3.67 N: 3.92 found C: 47.25 H: 3.71 N: 3.78

6-(Benzylamino)-2-fluoro-3-iodobenzaldehyde (52a)

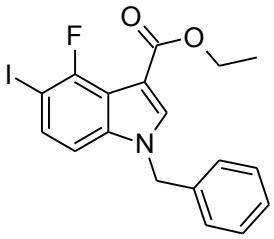


Activated MnO_2 (1.7 g, 20 mmol) was added to a solution of the benzyl alcohol **51a** (700 mg, 1.96 mmol) in 15 mL DCM. The reaction mixture was stirred overnight, filtered, and the solvent was evaporated under reduced pressure. Flash chromatography (PE/EA = 95:5) provided the desired benzaldehyde as a colorless solid **52a** in 61 % (420 mg, 1.18 mmol) yield.

M.p. 113-115 °C

^{1}H-NMR (200 MHz, $CDCl_3$) δ 10.31 (s, 1H), 9.23 (s, 1H), 7.55 (t, J = 7.4 Hz, 1H), 7.53-7.30 (m, 5H), 6.31 (d, J = 9.1 Hz, 1H), 4.74 (d, J = 5.7 Hz, 2H); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 189.03 (J = 11.8 Hz), 165.03 (J = 252.5 Hz), 151.82 (J = 3.4 Hz), 145.13 (J = 5.4 Hz), 137.35, 128.89, 127.61, 126.91, 110.12 (J = 3.6 Hz), 108.72 (J = 11.0 Hz), 60.88 (J = 26.1 Hz), 46.88; ^{19}F-NMR (200 MHz, $CDCl_3$) δ -102.27; HRMS $C_{14}H_{11}FINO$ $[M+H]^+$ calculated 355.9942 found 355.9943; E.A. calculated C: 47.08 H: 3.67 N: 3.92 found C: 47.25 H: 3.71 N: 3.78

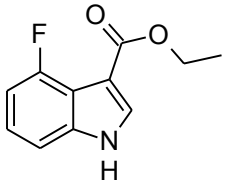
Ethyl 1-benzyl-4-fluoro-5-iodo-1*H*-indole-3-carboxylate (53), according to Levesque et al.[193]



BF_3OEt_2 (0.67 ml, 2.5 mmol) was added dropwise to a solution of the benzaldehyde **52a** (865 mg, 2.44 mmol) and ethyl diazoacetate (1.4 g, 12.2 mmol) in 15 mL DCM at 0 °C. The reaction mixture was stirred for 3 h at 0 °C before a saturated aqueous solution of $NaHCO_3$ was added. The aqueous phase was repeatedly extracted with DCM, the combined organic extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure. Flash chromatography of the crude mixture (PE/EA = 4:1, R_f = 0.33) provided the desired indole **53** as a colorless solid in 92 % (954 mg, 2.25 mmol) yield.

^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.49 (dd, J = 8.4 Hz, J = 5.4 Hz, 1H), 7.34-7.31 (m, 3H), 7.11 (d, J = 7.1 Hz, 2H), 6.87 (d, J = 8.6 Hz, 1H), 5.30 (s, 1H), 4.36 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 163.54, 154.94 (J = 251.2 Hz), 139.53 (J = 9.9 Hz), 135.75, 135.18, 132.67, 129.13, 128.42, 126.90, 115.12 (J = 22.0 Hz), 108.30 (J = 4.1 Hz), 107.09 (J = 4.3 Hz), 73.02 (J = 26.0 Hz), 60.35, 51.13, 14.44; ^{19}F-NMR (200 MHz, $CDCl_3$) δ -89.73, HRMS: $C_{18}H_{15}FINO_2$ $[M+H]^+$ calculated 424.0204 found 424.0204; E.A. calculated C:51.08 H: 3.57 N: 3.31 found C: 50.78 H: 3.59 N: 3.22

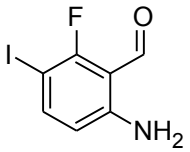
Ethyl 4-fluoro-1*H*-indole-3-carboxylate (56b), according to Murakami et al.[176]



$AlCl_3$ (96 mg, 0.72 mmol) was added to a solution of **53** (100 mg, 0.24 mmol) in 5 mL benzene. The reaction mixture was stirred for 2 h at room temperature before water was added. The aqueous phase was repeatedly extracted with DCM, the combined organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. Flash chromatography of the crude mixture provided the debenzylated and deiodinated indole as a yellowish solid **56b** in 78 % (40 mg, 0.19 mmol) yield.

^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.94 (s, 1H), 7.23 (d, J = 8.1 Hz, 1H), 7.17 (dd, J = 12.5 Hz, J = 7.7 Hz, 1H), 6.92 (t, J = 9.2 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 164.57, 156.32 (J = 249.8 Hz), 139.35 (J = 10.6 Hz), 132.37, 123.86 (J = 7.8 Hz), 114.02 (J = 20.2 Hz), 108.03 (J = 3.6 Hz), 107.90, 107.76, 60.30, 14.42 (J = 4.2 Hz); ^{19}F-NMR (200 MHz, $CDCl_3$) δ -112.76; HRMS: $C_{11}H_{10}FNO_2$ $[M+H]^+$ calculated 208.0787 found 208.0786 $[M+Na]^+$ calculated 230.0588 found 230.0588 ; E.A. calculated C: 63.76 H: 4.86 N: 6.76 found C: 63.35 H: 4.79 N: 6.59

6-Amino-2-fluoro-3-iodobenzaldehyde (52b), according to Satam et al.[197]

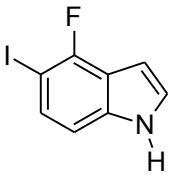


IBX (873 mg, 3.12 mmol) was added to a solution of **51a** (934 mg, 2.6 mmol) in 15 mL DMSO at room temperature. The reaction mixture was stirred for 30 min before water was added. The aqueous phase was extracted several times with Et_2O, the combined organic extracts were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. Flash chromatography of the crude mixture provided the aniline **52b** as a yellow solid in 67 % (450 mg, 1.7 mmol) yield.

^{1}H-NMR (200 MHz, DMSO) δ 10.12 (s, 1H), 7.62 (broad s, 2H), 7.56 (t, J = 8.3 Hz, 1H), 6.49 (d, J = 9.0 Hz, 1H); ^{13}C-NMR (200 MHz, DMSO) δ 188.64 (J = 10.6 Hz), 164.75 (J = 248.6 Hz), 152.71 (J = 4.1 Hz), 144.33 (J = 5.5 Hz), 115.41 (J = 3.3 Hz), 107.99 (J = 5.9 Hz), 60.97 (J = 26.0 Hz); ^{19}F-NMR (200 MHz, DMSO) δ -104.24; HRMS: C_7H_5FINO $[M-H]^+$ calculated 263.9316 found 263.9317; E.A. calculated: C: 31.72 H: 1.90 N: 5.29 found C: 31.80 H: 2.04 N: 5.21

4.6 Synthesis of the precursor of L-4-fluorotryptophan by linear synthesis

4-Fluoro-5-iodo-1*H*-indole (60), according to Schlosser et al.[69]

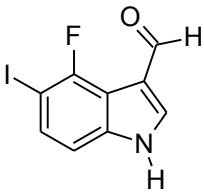


Analogously prepared as iodoindole **35** while starting from 4-fluoro-1-(triisopropylsilyl)indole (**2b**) giving the desired product as a colorless solid in 65 % yield.

M.p. 58 – 60 °C;

^{1}H-NMR ($CDCl_3$) δ 8.30 (broad s, 1H), 7.42 (dd, J = 8.4 Hz, J = 6.0 Hz, 1H), 7.14 (t, J = 2.7 Hz, 1H), 6.99 (d, J = 8.5 Hz, 1H), 6.61 (t, J = 2.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ 155.18 (J = 244.7 Hz), 138.32 (J = 10.8 Hz), 131.13, 124.68, 117.78 (J = 24.2), 109.17 (J = 3.7 Hz), 98.81 (J = 5.9 Hz), 68.30 (J = 23.4 Hz); ^{19}F-NMR ($CDCl_3$) δ -101.74; E.A. calculated: C: 36.81 H: 1.93 N: 5.37 found C: 37.64 H : 2.17 N: 5.14

4-Fluoro-5-iodo-1*H*-indole-3-carbaldehyde (61), according to Konas et al.[160]

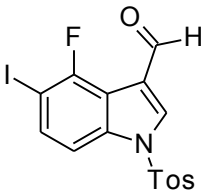


Analogously prepared as indolecarbaldehyde **36** but starting from 4-fluoro-5-iodo-1*H*-indole (**60**) giving the desired product as an off white solid in 89 % yield.

M.p. decompostition at 246-249 °C

^{1}H-NMR (DMSO) δ 12.60 (s, 1H), 10.02 (d, J = 3.3 Hz, 1H), 8.30 (d, J = 2.9 Hz, 1H), 7.60 (dd, J = 8.6 Hz, J = 5.8 Hz, 1H), 7.23 (d, J = 8.5 Hz, 1H); ^{13}C-NMR (DMSO) δ 184.07 (J = 1.7 Hz), 155.09 (J = 245.1 Hz), 140.10 (J = 11.3 Hz), 136.79, 132,81, 116.84 (J = 6.5 Hz), 113.81 (J = 24.6 Hz), 111.70 (J = 3.7 Hz), 73.23 (J = 24.4 Hz); ^{19}F-NMR (DMSO) δ -92.94; HRMS: C_9H_5FINO $[M-H]^+$ calculated 289.9473 found 289.9477; E.A.: calculated: C: 37.40 H: 1.74 N: 4.85 found: C: 37.86 H:1.84 N: 5.22

4-Fluoro-5-iodo-1-tosyl-1*H*-indole-3-carbaldehyde (62), according to Konas et al.[160]

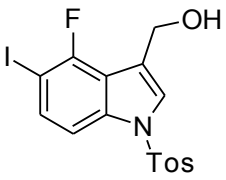


Analogously prepared as indolecarbaldehyde **37** while starting from 4-fluoro-5-iodo-1*H*-indole-3-carbaldehyde (**61**) giving the desired product as a colorless solid in 74 % yield.

M.p. decomposition at 148-153 °C.

^{1}H-NMR ($CDCl_3$) δ 10.02 (s, 1H), 8.65 (d, 1H, J = 6.2 Hz), 8.19 (s, 1H), 7.83 (d, 2H, J = 8.1 Hz), 7.69 (d, 1H, J = 8.3 Hz), 7.32 (d, 2H, J = 8.1 Hz), 2.39 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ 184.70, 159.51 (J = 242.0 Hz), 146.63, 136.51 (J = 3.2), 135.25 (J = 11.2 Hz), 133.71, 132.60 (J = 2.8 Hz), 130.47, 127.13, 124.16 (J = 2.0 Hz), 120.86 (J = 0.9 Hz), 100.71 (J = 31.3 Hz), 78.44 (J = 27.3 Hz), 21.63 ; ^{19}F-NMR ($CDCl_3$) δ -89.70;HRMS: $C_{16}H_{11}FINO_3S$ $[M+H]^+$ calcd. 443,9561 found 443,9561; E.A.: calcd. C: 43,36 H: 2,50 N: 3,16 found C: 43,05 H: 2,59 N: 3,11

[4-Fluoro-5-iodo-1-tosyl-1*H*-indol-3-yl]methanol (63), according to Konas et al.[160]



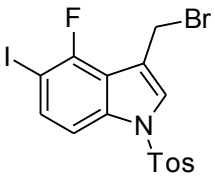
Analogously prepared as tosylate **38** by starting from 4-fluoro-5-iodo-1-tosyl-1*H*-indole-3-carbaldehyde (**62**) giving the desired product as a colorless solid in 97 % yield.

M.p. 131-132 °C

^{1}H-NMR (DMSO) δ 7.87 (d, J = 8.4 Hz, 2H), 7.70 (dd, J = 8.6 Hz, J = 6.0 Hz, 1H), 7.63 (s, 1H), 7.61 (d, J = 8.7 Hz, 1H), 7.40 (d, J = 8.2 Hz, 2H), 5.26 (t, J = 5.4 Hz, 1H), 4.63 (d, J = 5.3 Hz), 2.33 (s, 3H); ^{13}C-NMR (DMSO) δ 154.63 (J = 244.7 Hz), 145.94, 136.70 (J = 9.8 Hz), 134.33, 133.73, 130.41, 126.79, 124.42, 121.74 (J = 4.8 Hz), 118.01 (J = 23.3 Hz),

111.63 (J = 3.8 Hz), 74.86 (J = 23.9 Hz), 55.53 (J = 1.8 Hz), 21.04; ^{19}F-NMR (DMSO) δ -100.88; HRMS: $C_{16}H_{13}FINO_3S$ $[M+Na]^+$ calculated 467.9537 found 467.9539; E.A. calculated C: 43.16 H: 2.94 N: 3.15 found C: 43.40 H: 3.03 N: 3.15

[3-(Bromomethyl)-4-fluoro-5-iodo-1-tosyl]-1*H*-indole (64), according to Appel et al.[189]

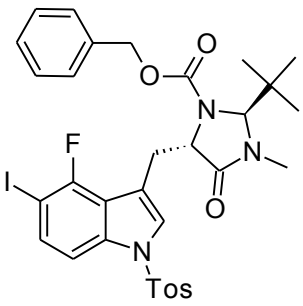


Analogously prepared as bromide **39** while starting from the alcohol **63** giving the desired product as a colorless solid 63 % yield.

M.p. decomposition at 166-168 °C

^{1}H-NMR (DMSO) δ 8.08 (s, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.75 (dd, J = 8.7 Hz, J = 6.0 Hz, 1H), 7.62 (d. J = 8.8 Hz, 1H), 7.42 (d, J = 8.2 Hz, 2H), 4.82 (s, 2H), 2.33 (s, 3H); ^{13}C-NMR (DMSO) δ 154.27 (J = 246.8 Hz), 146.16, 136.29 (J = 9.1 Hz), 134.91, 133.28, 130.41, 126.74, 124.28, 117.37 (J = 22.8 Hz), 117.00 (J = 3.7 Hz), 111.57 (J = 3.9 Hz), 75.62 (J = 24.1 Hz), 55.39 (J = 1.8 Hz), 20.94; ^{19}F-NMR (DMSO) δ -102.l3; HRMS: $C_{16}H_{12}BrFINO_2S$ $[M-Br]^-$ calculated 427.9617 found 427.9636; E.A. calculated C: 37.82, H: 2.38 N: 2.73 found C: 38.12 H: 2.38 N: 2.64

Benzyl (2S,5S)-2-*tert*-butyl-5-[(4-fluoro-5-iodo-1-tosyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimidazolidine-1-carboxylate (65)

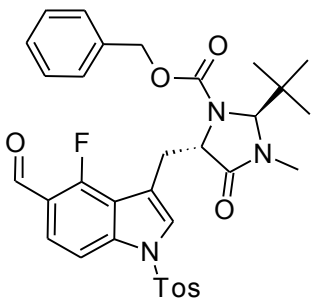


Analogously prepared as **40** but starting from the bromide **64** giving the desired product as a colorless solid in 73 % yield.

M.p. 83 – 86 °C

^{1}H-NMR ($CDCl_3$) δ 7.73 (broad s, 2H), 7.52 (dd, J = 8.7 Hz, J = 5.7 Hz, 1H), 7.47 (d, J = 8.6 Hz, 1H), 7.25 (broad s, 2H), 7.15-6.60 (broad m, 6H), 5.18 (broad s, 1H), 4.84 (d, J = 6.1 Hz, 2H), 4.38 (s, 1H), 3.76 (broad s, 1H), 3.40 (d, J = 14.4 Hz, 1H), 3.06 (s, 3H), 2.32 (s, 3H), 1.02 (s, 9H); ^{13}C-NMR ($CDCl_3$) δ 171.12, 155.39 (J = 248.9 Hz), 145.47, 136.54 (J = 9.1 Hz), 134.86, 134.68, 134.23, 130.06, 128.48, 128.33, 128.27, 127.07, 122.15, 120.23 (J = 21.2 Hz), 115.04 (J = 3.0 Hz), 111.15 (J = 3.8 Hz), 81.21, 73.83 (J = 24.4 Hz), 67.61, 60.41, 58.28, 40.85, 32.13, 26.30, 21.08, 21.60; ^{19}F-NMR ($CDCl_3$) δ -100.94; HRMS: $C_{33}H_{32}FIN_3O_5S$ $[M+Na]^+$ calculated 740.1061 found 740.1053; E.A. calculated C: 53.56 H: 4.64 N: 5.86 found C: 53.13 H: 4.58 N: 5.39

Benzyl (2S,5S)-2-*tert*-butyl-5-[(4-fluoro-5-formyl-1-tosyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimidazolidine-1-carboxylate (66), according to Boymond et al.[191]

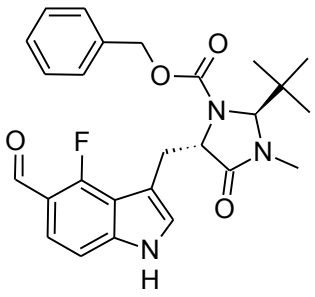


Analogously prepared as **41** by starting from **65** giving the desired product as a colorless solid in73 % yield.

M.p. 73-75 °C

^{1}H-NMR ($CDCl_3$) δ 10.34 (s, 1H), 7.81-7.71 (m, 4H), 7.31 (d, J = 7.7 Hz, 2H), 7.02-6.87 (m, 6H), 5.16 (s, 1H), 4.93 (s, 2H), 4.45 (s, 1H), 4.16 (d, J = 7.1 Hz, 1H), 3.53 (d, J = 17.2 Hz, 1H), 3.05 (s, 3H), 2.37 (s, 3H), 1.06 (s, 9H); ^{13}C-NMR ($CDCl_3$) δ 186.55 (J = 7.9 Hz), 171.02, 160.01 (J = 263.5 Hz), 145.83, 140.02 (J = 11.0 Hz), 134.90, 134.60, 130.19, 128.41, 128.29, 127.15, 123.93, 119.48 (J = 16.8 Hz), 118.81 (J = 5.2 Hz), 116.01, 109.89 (J = 3.5 Hz), 81.25, 67.62, 40.83, 32.08, 26.31, 21.63; ^{19}F-NMR ($CDCl_3$) δ -129.12, HRMS: $C_{33}H_{34}FN_3O_6S$ $[M+Na]^+$ calculated 642.2044 found 642.2030, E.A: calculated: C: 63.96 H: 5.53, N: 6.78 found C: 63.91 H: 5.63 N: 6.48

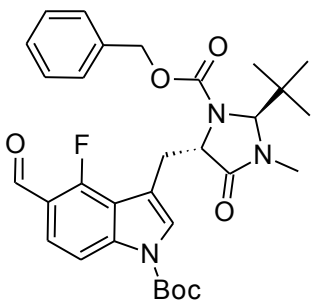
Benzyl (2S,5S)-2-*tert*-butyl-5-[(4-fluoro-5-formyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxo-imidazol-idine-1-carboxylate (67), according to Yasuhara et al.[170]



3.5 mL of 1.0 M solution of TBAF in THF were added to a solution of 432 mg (0.7 mmol) **66** in 10 mL THF. The resulting mixture was stirred at room temperature for 3 h before the solvent was evaporated *in vacuo*. Flash chromatography of the crude mixture gave the desired product as a colorless solid in 42 % (140 mg, 0.3 mmol)

^{1}H-NMR (DMSO) δ 11.53 (s, 1H), 10.27 (s, 1H), 7.48 (dd, J = 8.4 Hz, J = 6.2 Hz, 1H), 7.27 (d, J = 8.5 Hz, 1H), 7.19-7.14 (m, 3H), 7.08 (d, J = 7.2 Hz, 2H), 6.72 (s, 1H), 5.19 (s, 1H), 5.00 (d, J = 12.3 Hz, 1H), 4.79 (d, J = 12.3 Hz, 1H), 4.50 (d, J = 4.4 Hz, 1H), 3.97-3.94 (broad s, 1H), 3.38 (d, J = 15.4 Hz, 1H), 2.93 (s, 3H), 0.93 (s, 9H); ^{13}C-NMR (DMSO) δ 187.15 (J = 7.5 Hz), 171.02, 160.84 (J = 262.5 Hz), 142.62 (J = 13.9 Hz), 136.29, 128.58, 128.30, 123.64, 120.46, 115.80 (J = 16.0 Hz), 115.21 (J = 5.3 Hz), 109.95, 109.23, 80.41, 66.90, 58.69, 31.84, 26.47; ^{19}F-NMR (DMSO) δ -130.47; HRMS: $C_{26}H_{28}FN_3O_4$ $[M+Na]^+$ calculated 488.1956 found 488.1992; E.A. calculated: C: 67.08 H: 6.06 N: 9.03 found C: 65.20 H: 5.92 N: 8.60

Benzyl (2S,5S)-2-*tert*-butyl-5-[(1-Boc-4-fluoro-5-formyl-1*H*-indol-3-yl)methyl]-3-methyl-4-oxoimidazolidine-1-carboxylate (68), according to de Koning et al.[159]



60 mg (0.27 mmol) Boc_2O were added to a solution of **67** (110 mg, 0.23 mmol) and DMAP (3 mg, 24 µmol) in 5 ml THF. The resulting mixture was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure. Purification of the crude mixture by flash chromatography (PE/EA = 7:3, R_f = 0.5) yielded the desired product as a colorless solid (86 %, 112 mg, 0.20 mmol).

M.p.: 133 – 136 °C

^{1}H-NMR ($CDCl_3$) δ 10.37 (s, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.74 (dd, J = 12.7 Hz, J = 6.5 Hz, 1H), 7.39-7.03 (m, 5H), 6.98 (s, 1H), 5.14-4.89 (m, 3H), 4.49 (d, J = 1.2 Hz, 1H), 4.03 (d, J = 16.8 Hz, 1H), 3.59 (d, J = 17.2 Hz), 3.11 (s, 3H), 1.58 (s, 9H), 0.99 (s, 9H); ^{13}C-NMR ($CDCl_3$) δ 187.45 (J = 6.7 Hz), 170.92, 160.64 (J = 261.3 Hz), 148.45, 140.51 (J = 11.5 Hz), 136.25, 128.33 (J = 5.3 Hz), 124.61, 123.23, 118.78, 118.53, 118.41, 114.50 (J = 3.0 Hz), 111.96, 85.51, 80.79, 66.83, 58.09, 31.99, 27.99, 26.38; ^{19}F-NMR ($CDCl_3$) δ -130.61; HRMS $C_{31}H_{36}FN_3O_4$ $[M+Na]^+$ calculated 588.2480 found 588.2461; E.A. calculated C: 65.83 H: 6.42 N: 7.43 found C: 64.58 H: 6.39 N: 7.32

4.7 Radiochemistry

4.7.1 Preparation of tetrabutylammonium [^{18}F]fluoride (TBA^{18}F)

N.c.a. [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction via bombardment of an isotopically enriched [^{18}O]water target with 17 MeV protons at the JSW cyclotron BC 1710 (FZ Jülich).[206] An aliquot of the [^{18}F]fluoride solution was added to 150 µL (18 µmol) of a 0.12 M tetrabutylammonium bicarbonate solution ($TBAHCO_3$).[31] After adding 1.0 mL of dry MeCN by a syringe, azeotropic distillation under a steam of argon at 75 °C and 600 mbar was performed. The azeotropic evaporation was repeated twice. Afterwards the vial was evacuated for about 5 min at 10 – 20 mbar and flushed again with argon.

4.7.2 General procedure for the radiosynthesis of 1-benzyl-[^{18}F]fluoro-1*H*-indoles ([^{18}F]1-4b) by conventional heating

A solution of 20 µmol of the corresponding precursor (**1e**, **2e**, **3e** or **4e**) in 1.0 mL DMF was added to the vial containing the dry TBA^{18}F with a typical activity of 15 to 30 MBq. The reaction mixture was heated to the desired temperature (usually between 60 and 160 °C) for typically 15 min. After labeling the reaction solution was diluted with 10.0 mL water and passed through a previously conditioned LiChrolut RP-18e cartridge (Merck, Germany). Afterwards, the fixed labeled compound was eluted with 2.0 ml acetonitrile into a second vial followed by the evaporation of the solvent. A solution containing Wilkinson's catalyst (2 – 4 eq.) in 1.0 mL of the desired solvent (benzonitrile or dioxane) was added and the mixture was heated to 150 °C for 20 min.

4.7.3 General procedure for the radiosynthesis of 1-benzyl-[^{18}F]fluoro-1*H*-indoles ([^{18}F]1-4b) by microwave heating

A solution of 20 µmol of the corresponding precursor in 1.0 mL DMF was added to the vial containing the dried TBA^{18}F with a typical activity of 15 to 30 MBq. The reaction mixture was heated with microwaves of the desired energy (usually between 30 and 140W) during 1 min. After labeling the solution was diluted with 10.0 mL water and passed through a previously conditioned LiChrolut RP-18e cartridge (Merck, Germany). Afterwards the fixed labeled compound was eluted with 2.0 ml acetonitrile into a second vial followed by the evaporation of the solvent. A solution containing Wilkinson's catalyst (2 – 4 eq.) in 1.0 mL of the desired solvent (benzonitrile or dioxane) was added and the mixture was heated with 100 W microwaves for 2 min.

4.7.4 General procedure for the radiosynthesis of L-4-[^{18}F]fluorotryptophan ([^{18}F]70) by conventional heating

A solution of 8-9 µmol of the precursor **68** in 1.0 mL DMF was added to the vial containing the dry TBA^{18}F with a typical activity of 200 – 300 MBq. The reaction mixture was heated to the desired temperature for typically 10 min. After labeling the reaction mixture was diluted with 1.0 mL acetonitrile and 9.0 mL water and passed through a previously conditioned LiChrolut RP-18e cartridge (Merck, Germany). Afterwards the cartridge was eluted with 2.0

mL acetonitrile, and the solvent was evaporated. A solution containing Wilkinson's catalyst (generally 2 – 4 eq.) in 1.0 mL benzonitrile was added and the reaction mixture was heated to the desired temperature for typically 20 min. The mixture was diluted with 1.0 mL of a solution of ethyl acetate in petroleum ether (5% EA/PE), and filtered through a silica cartridge (600-700 mg silica gel in a 3 mL polyethylene filtration tube) followed by elution with 9 mL of the same solvent mixture. Subsequently the desired compound was eluted with a solution of ethyl acetate in petroleum ether (60% EA/PE). The solvents were evaporated followed by the addition of 0.5 ml of concentrated hydrochloric acid and heating of the reaction mixture to the temperature of choice (110 – 160 °C) for usually 30 min.

4.7.5 General procedure for the radiosynthesis of L-4-[^{18}F]fluorotryptophan ([^{18}F]70) under microwave heating

A solution of 8-9 μmol of the precursor **68** in 1.0 mL DMF was added to a vial containing the dry TBA^{18}F with activities of usually 200 – 300 MBq. The reaction mixture was treated with microwaves of the desired energy (usually 30 - 80 W) for 1 min. After labeling the reaction mixture was diluted with 1.0 mL acetonitrile and 9.0 mL water and passed through a previously conditioned LiChrolut RP-18e cartridge (Merck, Germany). Afterwards the cartridge was eluted with 2.0 mL acetonitrile and the solvent was evaporated. A solution containing Wilkinson's catalyst (2 – 4 eq.) in 1.0 mL benzonitrile was added and the reaction mixture was heated with 100 W microwaves for 2 min. The mixture was diluted with 1.0 mL of a solution of ethyl acetate in petroleum ether (5% EA/PE), and filtered through a silica cartridge (600-700 mg silica gel in a 3 mL polyethylene filtration tube) followed by elution with 9 mL of the same solvent mixture. Subsequently the desired compound was eluted with a solution of ethyl acetate in petroleum ether (60% EA/PE). The solvents were evaporated followed by the addition of 0.5 ml of concentrated hydrochloric acid, and the reaction mixture was heated to temperatures between 110 and 160 °C for typically 30 min.

5. Summary and outlook

Aromatic α-[^{18}F]fluoroamino acids have been developed as potential tracers for different purposes in nuclear medicine. The most established of those amino acids for PET are 6-[^{18}F]fluoro-L-DOPA and *O*-[^{18}F]fluoroethyl-L-tyrosine ([^{18}F]FET) which are widely used for the diagnosis of cerebral and peripheral endocrine tumors. Furthermore, 6-[^{18}F]fluoro-L-DOPA is also used for the diagnosis of several central motor disorders that are due to striatal (mal-)functions.

Another interesting aromatic amino acid is tryptophan which has earlier been radiofluorinated by electrophilic approaches or secondary groups. On one hand, radiolabeled derivatives of tryptophan have been prepared in the past aiming at the measurement of serotonin synthesis, and they might be useful for the diagnosis of diseases that are connected with the serotonergic receptor system. On the other hand, radiofluorinated tryptophan might also be useful in tumor diagnosis, since recent studies have revealed that some types of tumors have highly elevated tryptophan consumption due to overexpression of the enzyme indoleamine dioxygenase.

Recently a nucleophilic approach made the radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine and 2-[^{18}F]fluoro-L-tyrosine available in three steps. In these studies the corresponding precursor was radiofluorinated by an isotopic ^{18}F-for-^{19}F exchange, followed by the removal of an activating aldehyde group by reductive decarbonylation, and a final step where hydrolysis of the protecting groups was performed. Based on this three-step procedure the radiosynthesis of L-4-[^{18}F]fluorotryptophan was realized in the present work.

Prior to the preparation of a suitable precursor for the radiosynthesis of L-4-[^{18}F]fluoro-tryptophan, the influence of the substitution pattern of several *ortho*- and *para*-substituted fluoro-1*H*-indolecarbaldehydes on an isotopic ^{18}F-exchange was investigated in order to find the optimal position of the fluorine that provides the highest RCY. Additionally, a reductive decarbonylation with Wilkinson's catalyst was examined on the fluoro-1*H*-indole-carbaldehydes in order to determine if the removal of the carbonyl function is easily possible for the selected precursor.

The precursors for the isotopic exchange on the fluoro-1*H*-indolecarbaldehydes **1-3** with an *ortho*-substitution pattern were prepared in a 4-step linear synthesis starting from corresponding fluoroindoles. The only precursor with a *para*-substitution pattern, 4-fluoro-1*H*-indole-7-carbaldehyde (**4**), was prepared by a Bartoli reaction.

The indole nitrogen of 4-, 6- and 7-fluoroindole was protected with a TIPS group followed by a formylation with *n*-BuLi and DMF. Subsequently, the TIPS group was removed with TBAF and the resulting unprotected fluoro-1*H*-indolecarbaldehydes (**1d**, **2d**, **3d**) were protected with different protecting groups (benzyl, methyl, Boc and tosyl) resulting in the corresponding precursors. For the preparation of the *para*-substituted indole, 1-bromo-4-fluoro-2-nitrobenzene was used in a Bartoli reaction for the synthesis of 7-bromo-4-fluoro-1*H*-indole (**4b**) which was then protected with a benzyl group and formylated with *n*-BuLi and DMF giving 1-benzyl-4-fluoro-1*H*-indole-7-carbaldehyde (**4e**) as precursor.

With respect to the isotopic exchange, the best RCY was obtained when the indole nitrogen of the precursors was protected with a benzyl group. A RCY of above 90 % was obtained for all 1-benzyl-fluoro-1*H*-indolecarbaldehydes (**2e**, **3e**, **4e**); except for 1-benzyl-6-fluoro-1*H*-indole-5-carbaldehyde (**1e**). It could only be labeled in ca. 60 %, RCY, but showed the best results regarding chemical stability. The radiofluorination reaction itself was carried out in DMF with $TBAHCO_3$ as anion activator. Protection with a methyl group provided a slightly lower RCY than the benzyl group while the Boc-protected compounds (**1g**, **2g**) gave a RCY of 6 % and 28 %, respectively. Labeling was not possible when the indole nitrogen was not protected or tosylated. The best results regarding RCY and chemical stability were obtained with 1-benzyl-4-fluoro-1*H*-indole-5-carbaldehyde (**2e**). Additionally, a microwave assisted procedure was developed for the isotopic exchange giving similar RCY as under conventional heating, but, however, in a much shorter reaction time of 1 min.

Furthermore, the decarbonylation reaction was performed on the 1-benzyl-fluoro-1*H*-indolecarbaldehydes **[^{18}F]1e**, **[^{18}F]2e**, **[^{18}F]3e** and **[^{18}F]4e** with Wilkinson's catalyst ($Rh(PPh_3)_3Cl$). When this reaction was done in dioxane, only a low RCY of about 6 % was obtained regardless the equivalents of catalyst, reaction temperature and time. With benzonitrile as solvent, 3 equivalents of $Rh(PPh_3)_3Cl$ and conventional heating of the reaction mixture to 150 °C for 20 min provided all 1-benzyl-fluoro-1*H*-indoles **[^{18}F]5**, **[^{18}F]6** and **[^{18}F]7** with a RCY of > 95 %. This reaction was also carried out with microwave assisted heating. Hereby 3 equivalents of $Rh(PPh_3)_3Cl$ and heating with microwaves of 100 W for 120 s gave a similar RCY compared to the reaction with conventional heating, whereas a shorter reaction time led to incomplete conversion of the starting material.

The synthesis of the precursor **43** for L-6-[^{18}F]fluorotryptophan was attempted following two principally different synthetic pathways: Via a build-up synthesis by a palladium mediated heteroannulation and alternatively by a linear synthesis.

For the build-up synthesis, the preparation of alkyne **15** and a functionalized 2-iodoaniline were necessary. The alkyne was prepared in three steps starting from propargyl alcohol by the introduction of a TMS group followed by tosylation and coupling with Z-BMI. The second molecule for the coupling should be one of the iodoanilines **11**, **12** or **13**, however, the preparation of none of those carbonyl activated iodoanilines was successful. Therefore, the coupling of 3-fluoro-4,6-diiodoaniline (**14**) with the alkyne **15** was examined in order to get a precursor for L-6-[^{18}F]fluorotryptophan. This resulted in a variety of products, but the tryptophan analogue **30** could not be isolated. For this reason the approach via this synthetic pathway was not further investigated.

The alternative preparation of the precursor **43** in a linear synthetic route started from 6-fluoroindole which was protected with a TIPS group followed by iodination in the 5- position with *sec*-BuLi and 1,2-diiodoethane. The TIPS group was removed with TBAF and a formyl group was introduced in the 3-position by a Vilsmeier-Haack reaction, resulting in the indole-carbaldehyde **36** which was protected and reduced to the corresponding alcohol **38a** with $NaBH_4$ and converted into the bromine **39a** by an Appel reaction. Subsequent coupling with Z-BMI and formylation gave the tosyl protected precursor **41**. The exchange of the tosyl to the benzyl protecting group was further performed by deprotection with TBAF and benzylation with BnBr which resulted in the final precursor **43** for L-6-[^{18}F]fluorotryptophan. The overall yield of the synthesis of this precursor was about 8 %.

As mentioned above, the radiosynthesis of L-6-[^{18}F]fluorotryptophan was performed in three steps: isotopic exchange, reductive decarbonylation and the removal of the protecting groups. For the isotopic exchange of **43** the optimum conditions were found with $TBAHCO_3$ as anion activator, DMF as solvent and at 150 °C. Hereby a RCY of about 9 % was obtained, while the formation of a diastereomer was observed. The ratio between the L- and D- diastereomer was about 70:30. The decarbonylation reaction gave a RCY of ca. 11 % with 3 equivalents $Rh(PPh_3)_3Cl$, benzonitrile as solvent and microwave heating with 100 W microwaves for 2 min. The removal of the protecting groups, however, could not be accomplished since the removal of the benzyl group on the indole nitrogen proved impossible. Any tested attempt for that resulted in decomposition of the decarbonylated precursor **[^{18}F]44**.

The synthesis of the precursor of the positional isomer L-4-[^{18}F]fluorotryptophan (**68**) was also carried out in two generally different ways. It was attempted by a build-up synthesis based on an [1,2]-aryl shift condensation as well as by a linear synthetic pathway similar to the one described above for the synthesis of the precursor **43**.

For the [1,2]-aryl shift condensation ethyl diazoacetate was necessary, which is commercially available, and 6-(benzylamino)-2-fluoro-3-iodobenzaldehyde (**52**) which was prepared in a linear six step synthesis starting from 2-amino-6-fluoro-benzoic acid (**46**). The first step thereby was a cyclisation with triphosgene followed by an esterification which was accomplished in MeOH with DMAP giving the aniline **48**. In the next step an iodination was performed with Ag_2SO_4 and elemental iodine, followed by a reductive amination with $NaBH_3CN$ and benzaldehyde. The reduction to the benzyl alcohol **51a** was carried out with DIBAL in THF, followed by an oxidation with MnO_2 giving the desired aminobenzaldehyde **52a**. An aryl shift condensation of ethyl diazoacetate and the aminobenzaldehyde **52a**, performed with $BF_3{\cdot}OEt_2$ in DCM, gave the *N*-benzylindole **53**. The reduction of the ethyl ester to the alcohol followed by a bromination through an Appel reaction, however, was not successful due to decomposition of the *N*-benzylindole **53**. Also, debenzylation of **53** with $AlCl_3$ led to deiodinated **56b** which made a formylation at a later stage impossible. Alternatively, formylation and protection of **53** prior to debenzylation resulted again in decomposition of the starting material which made a synthesis of a suitable precursor (**68**) for L-4-[^{18}F]fluorotryptophan impossible via this approach.

The synthesis of precursor **68** was therefore performed according to the linear synthesis described for the preparation of **43**. Only the protecting group on **68** was changed from benzyl to Boc, since the 1-Boc-4-fluoro-1*H*-indole-5-carbaldehyde (**2g**) provided a RCY of 28 % for the isotopic exchange and the Boc group can be removed under milder conditions than the benzyl group. In summary, the synthesis of precursor **68** was successfully realized with 8 % overall yield following an 11 step linear synthetic procedure.

The first attempt of the isotopic exchange on **68** was done under the optimum conditions obtained for **2g** leading to a RCY of ca. 19 %. After optimization, however, a RCY of about 51 % was obtained by heating the reaction mixture to 80 °C for 10 min while $TBAHCO_3$ was used as anion activator. Also here, the microwave assisted reaction was investigated, but leading to a somewhat lower RCY of about 41 % under optimum conditions.

The decarbonylation reaction was carried out under the optimum conditions obtained for that of 1-benzyl-fluoro-1*H*-indolecarbaldehydes and optimized regarding the molar ratio of $Rh(PPh_3)_3Cl$, solvent and reaction time. The optimum conditions with conventional heating were found to be 3 equivalents of $Rh(PPh_3)_3Cl$ and keeping the reaction mixture at 150 °C for 20 min. Those conditions provided a RCY of about 45 %. When this reaction was performed with microwave heating, this time, an excellent RCY of ca. 75 % was obtained under the

optimum conditions of 3 equivalents of $Rh(PPh_3)_3Cl$ and heating with 100 W microwaves for 2 min.

The final step in the radiosynthetic pathway was the hydrolysis which was done with conc. HCl by heating the reaction mixture to 150 °C for 30 min giving a RCY of about 34 %. In this case, a side product was formed that was not subject to incomplete hydrolysis but not further characterized.

After optimization of all three reaction steps the desired L-4-[^{18}F]fluorotryptophan could be isolated in a total RCY of about 13 % and an enantiomeric purity of > 99 % in about 115 min. All reactions were carried out under developmental conditions with low starting activities of around 250 MBq. The obtained specific activity under those conditions was always > 70 MBq/mmol which is in the area of specific activities for standard electrophilic fluorinations, but is expected to increase considerably when higher starting activities are applied that are commonly used for routine production.

On the basis of the obtained results with the radiosynthesis of L-4-[^{18}F]fluorotryptophan several suggestions for optimizations can be made. The step with the lowest RCY was the hydrolysis step. This was probably due to the harsh reaction conditions that are necessary for the cleavage of the BMI group. During the synthesis of the precursor and the radiosynthesis it proved that the precursor **68** is highly sensitive towards elevated temperatures. Hence, the introduction of a chiral protecting group for the amino acid function is desirable that can be hydrolyzed under less aggressive conditions. A suggestion for the preparation of such a precursor would be the introduction of the chiral center with Schöllkopf's bislactimether which can be cleaved under much milder conditions. The amine function could then be protected with a trityl group that can also be hydrolyzed under very mild conditions within a short time.

Furthermore, it would be interesting to test if a Baeyer-Villiger Oxidation is possible on the labeled compound **[^{18}F]68** under reaction conditions where L-4-[^{18}F]fluoro-5-hydroxy-tryptophan is expected to be the major product after hydrolysis of the protecting groups. This would provide another potentially very interesting new radiotracer, since L-5-hydroxytryptophan is an important intermediate in the serotonergic pathway.

6. References

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Abbreviations

5-FPTRP	5-(-3-fluoropropyl)-DL-tryptophan
5-HT	5-hydroxytyramine
AADC	aromatic amino acid decarboxylase
AcOH	acetic acid
Ag_2SO_4	silver sulfate
$AlCl_3$	aluminum chloride
ATP	adenosine triphosphate
BF_3OEt_2	trifluoroboroetherate
BGO	bismut-germanate
BnBr	benzyl bromide
Boc_2O	di-tert-butyl dicarbonate
Boc-BMI	(S)-(−)-1-Boc-2-tert-butyl-3-methyl-4-imidazolidinone
Bq	Becquerel
BuLi	butyllithium
c.a.	carrier added
cAMP	cyclic adenosine monophosphate
CBr_4	tetrabromomethane
CH	conventional heating
CNS	central nervous system
$COCl_2$	phosgene
CT	computed tomography
DCM	dichloromethane
DIBAL	diisobutylaluminium hydride

DMAP	*N,N*-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMP	Dess-Martin periodane
DMSO	dimethylsulfoxide
DOPA	dihydroxy phenylalanine
e.e.	enantiomeric excess
EA	ethyl acetate
EDA	ethyl diazoacetate
EOB	end of bombardment
Et_2O	diethyl ether
Et_3N	triethylamine
EtOH	ethanol
FDG	2-fluorodeoxyglucose
FEHTP	5-(2-fluoroethoxy)-L-tryptophan
H_2O	water
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
I_2	iodine
IBX	2-iodoxybenzoic acid
IDO	indoleamin dioxygenase
IP_3	inositol triphosphate
i-PrMgBr	isopropyl magnesium bromide
i-PrMgCl	isopropyl magnesium chloride
KI	potassium iodide
KOH	potassium hydroxide
LDA	lithium diisopropylamine

LSO	lutetium-yttrium-oxide-ortho-oxosilicate
M.p.	melting point
MeCN	acetonitrile
MeI	iodomethane
MeOH	methanol
MH	microwave heating
MnO_2	manganese dioxide
MRI	magnetic resonance tomography
n.c.a.	no carrier added
Na_2SO_4	sodium sulfate
$NABH_3CN$	sodium cyanoborohydride
$NaBH_4$	sodium borohydride
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
$NaIO_4$	sodium periodate
NH_4HCO_2	ammonium formate
NMR	nuclear magnetic resonance
PE	petroleum ether
PET	positron-emission-tomography
$POCl_3$	phosphorous oxychloride
PPh_3	triphenylphosphine
PPi	pyrophosphate
PRPP	phosphor-ribosylpyrophosphate
PSR	protein synthesis rate
PTC	phase transfer catalyst
RCY	radiochemical yield

$Rh(PPh_3)_3Cl$	Wilkinson's catalyst
RT	room temperature
SPECT	single-photon-emission-tomography
$t_{1/2}$	half life
TBA	tetrabutylammonium
TBAF	tetrabutylammonium fluoride
TDO	tryptophan dioxygenase
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Tos-Cl	tosyl chloride
TPH	tryptophan hydroxylase
Z-BMI	(S)-1-Z-2-tert-Butyl-3-methyl-4-imidazolidinone
$ZnCl_2$	zinc chloride
α-MTrp	alpha-methyltryptophan

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